



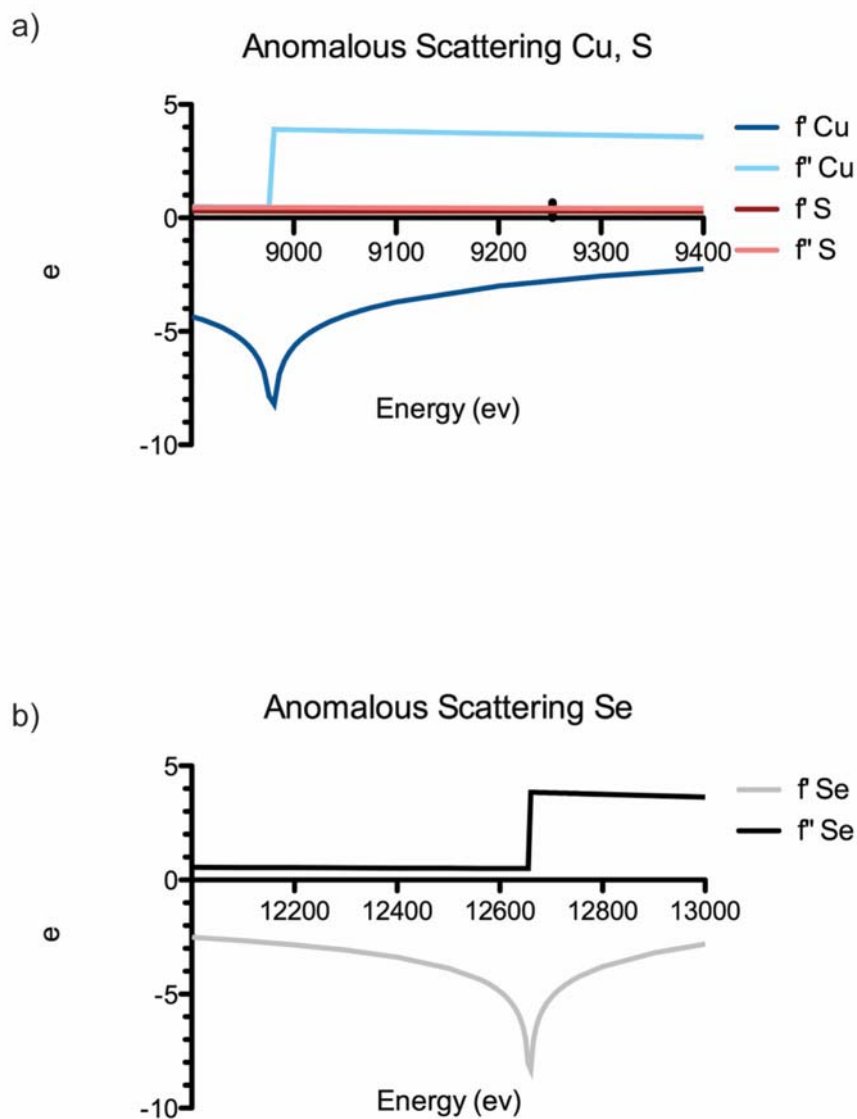
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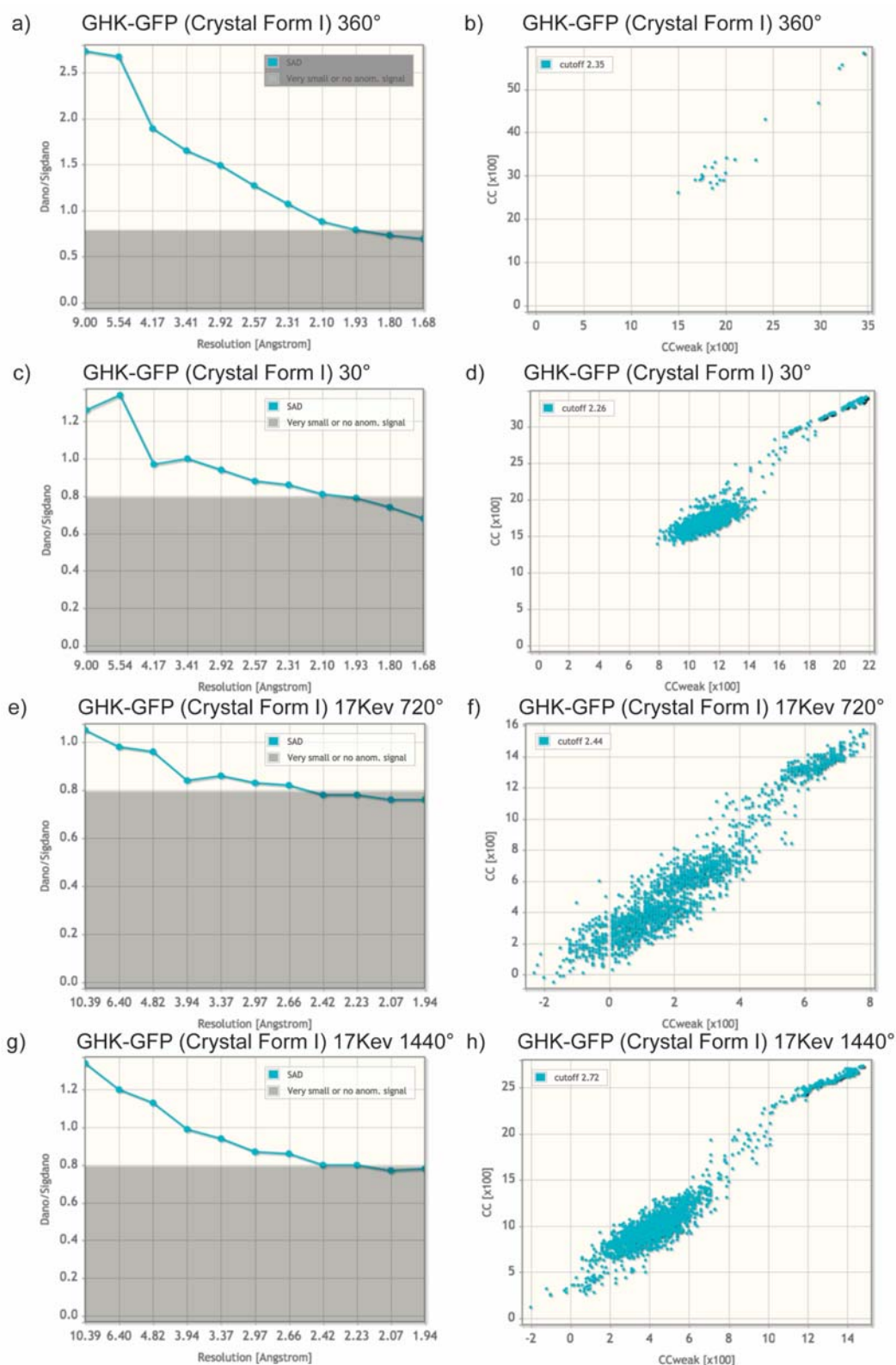
**Supporting information for article:**

**The copper(II)-binding tripeptide GHK, a valuable  
crystallization and phasing tag for macromolecular  
crystallography**

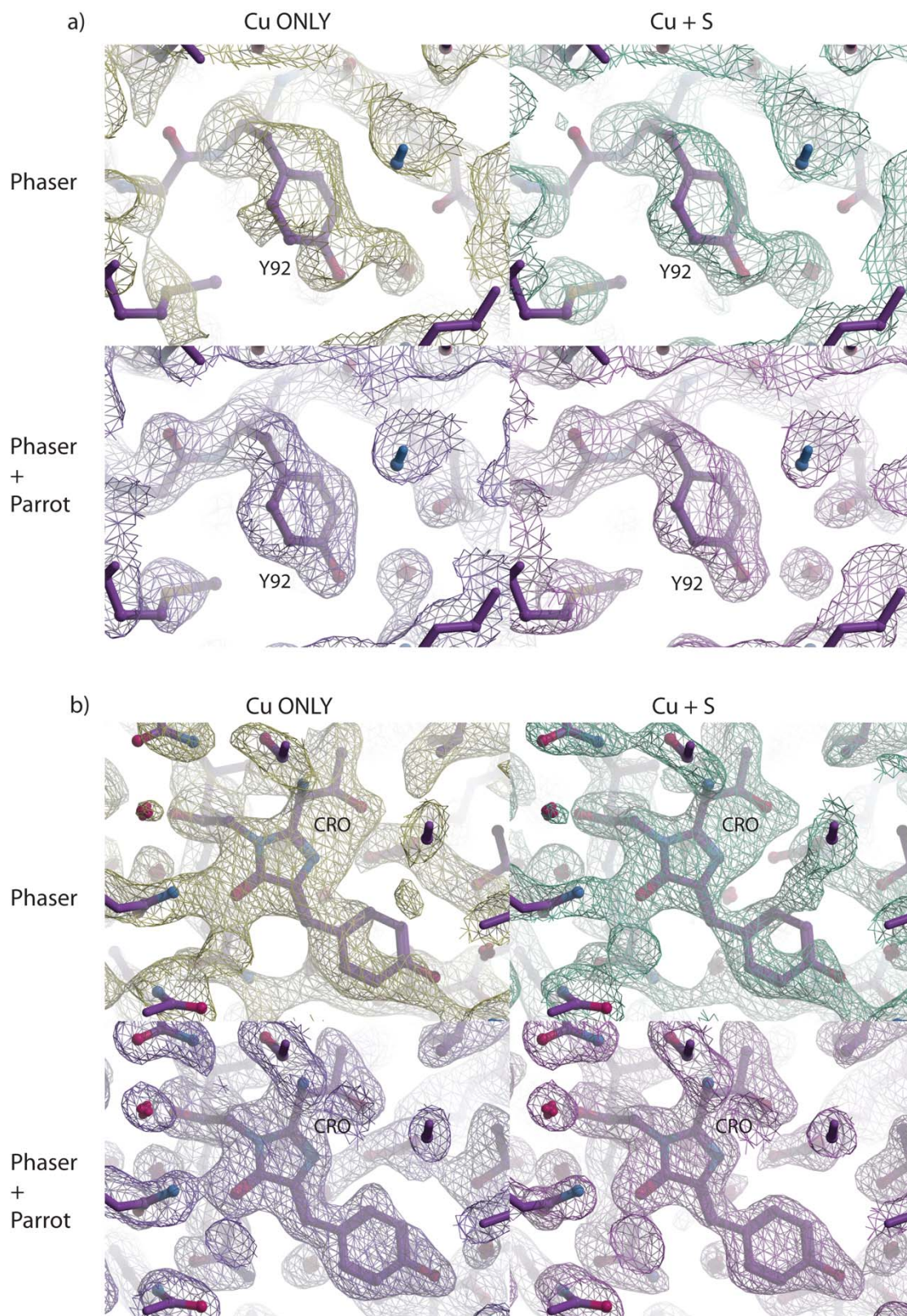
**Alexander Mehr, Fabian Henneberg, Ashwin Chari, Dirk Görlich and  
Trevor Huyton**



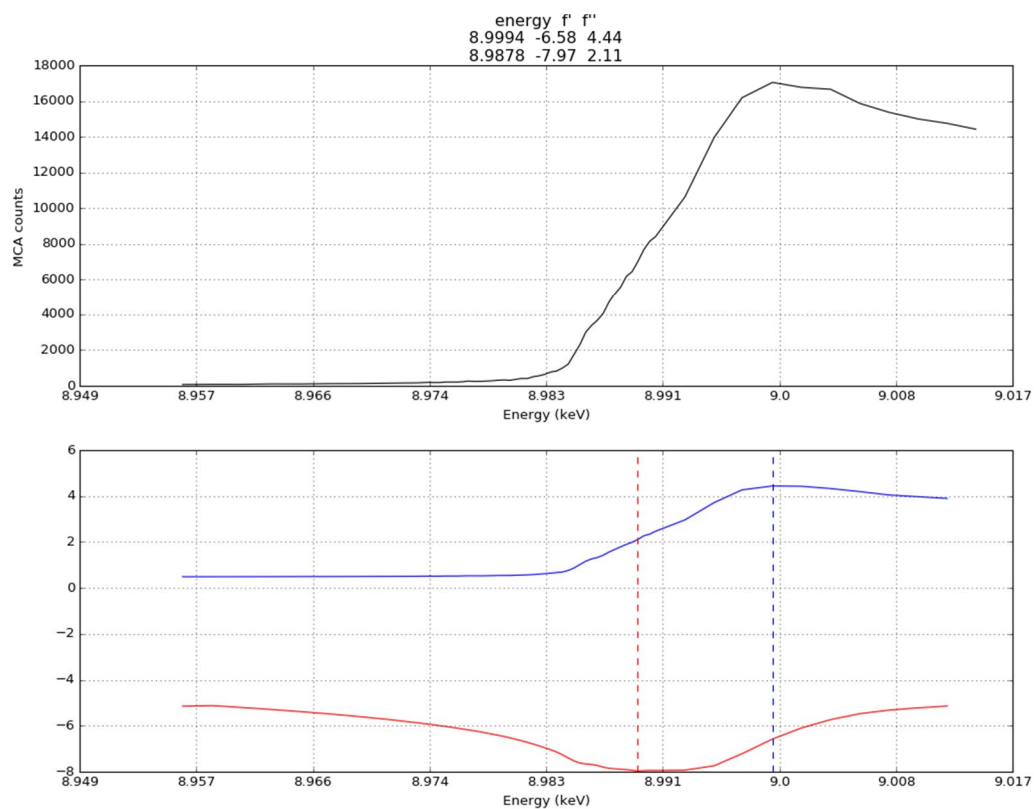
**Figure S1** a) The theoretical anomalous scattering for Cu and S (between 8900 and 9400 eV) is 3.69  $\epsilon$  at 9252.5 eV (1.34  $\text{\AA}$ ) b) Comparison with the scattering of 3.84  $\epsilon$  for selenium at its K-edge 12660.0 eV (0.9793  $\text{\AA}$ ). Data points were plotted from the webserver <http://skuld.bmsc.washington.edu/scatter/>



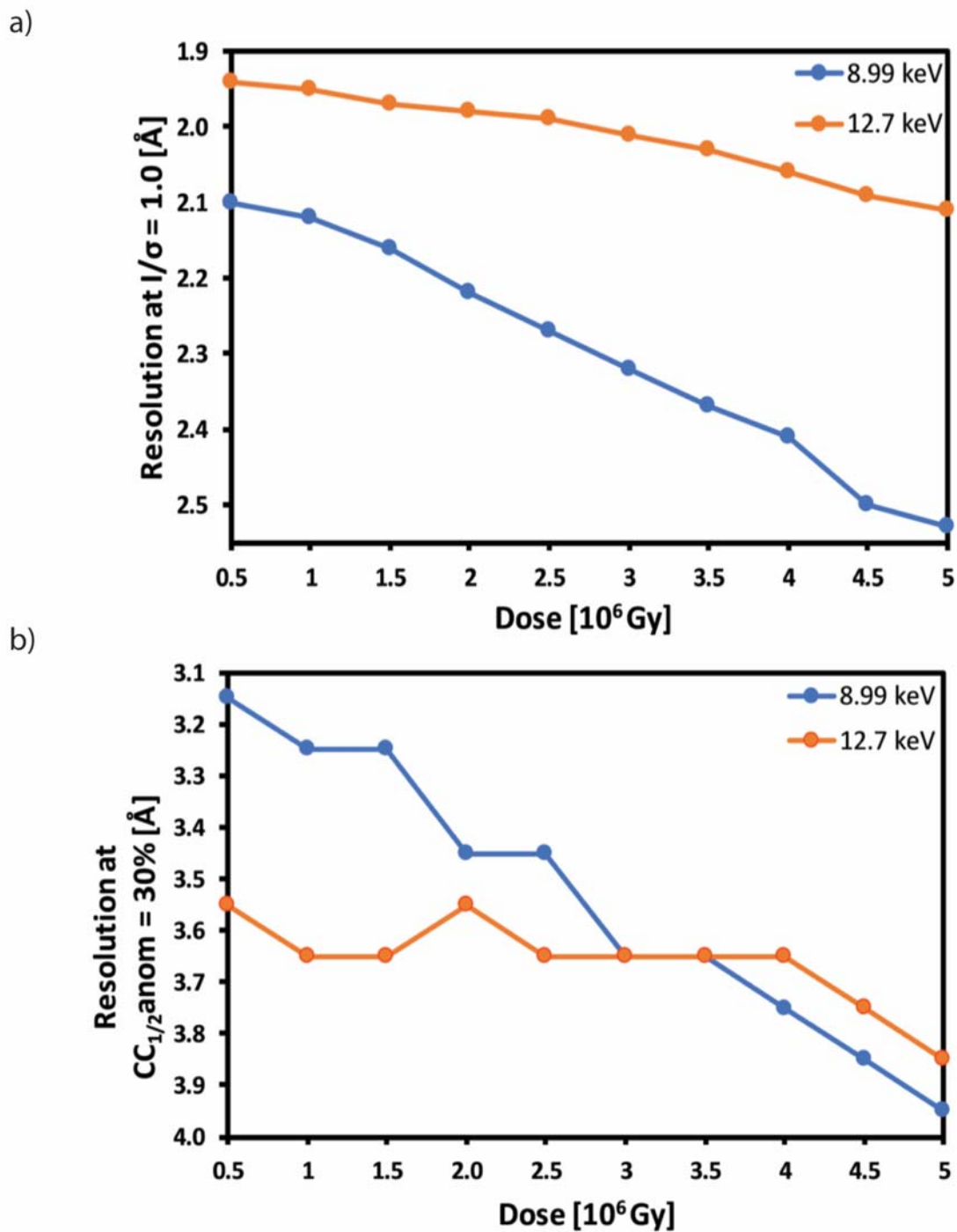
**Figure S2** Output from *CRANK2* pipeline run via *CCP4* online (<https://ccp4serv7.rc-harwell.ac.uk/ccp4online>) for GHK-GFP crystal form I. Panels a,c,e,g; Anomalous signal estimation output from *SHELXC*. Panels b,d,f,h; Profiles of *SHELXD* correlation coefficients (CC) for substructure determination. Results are shown for the datasets given in Table 1.



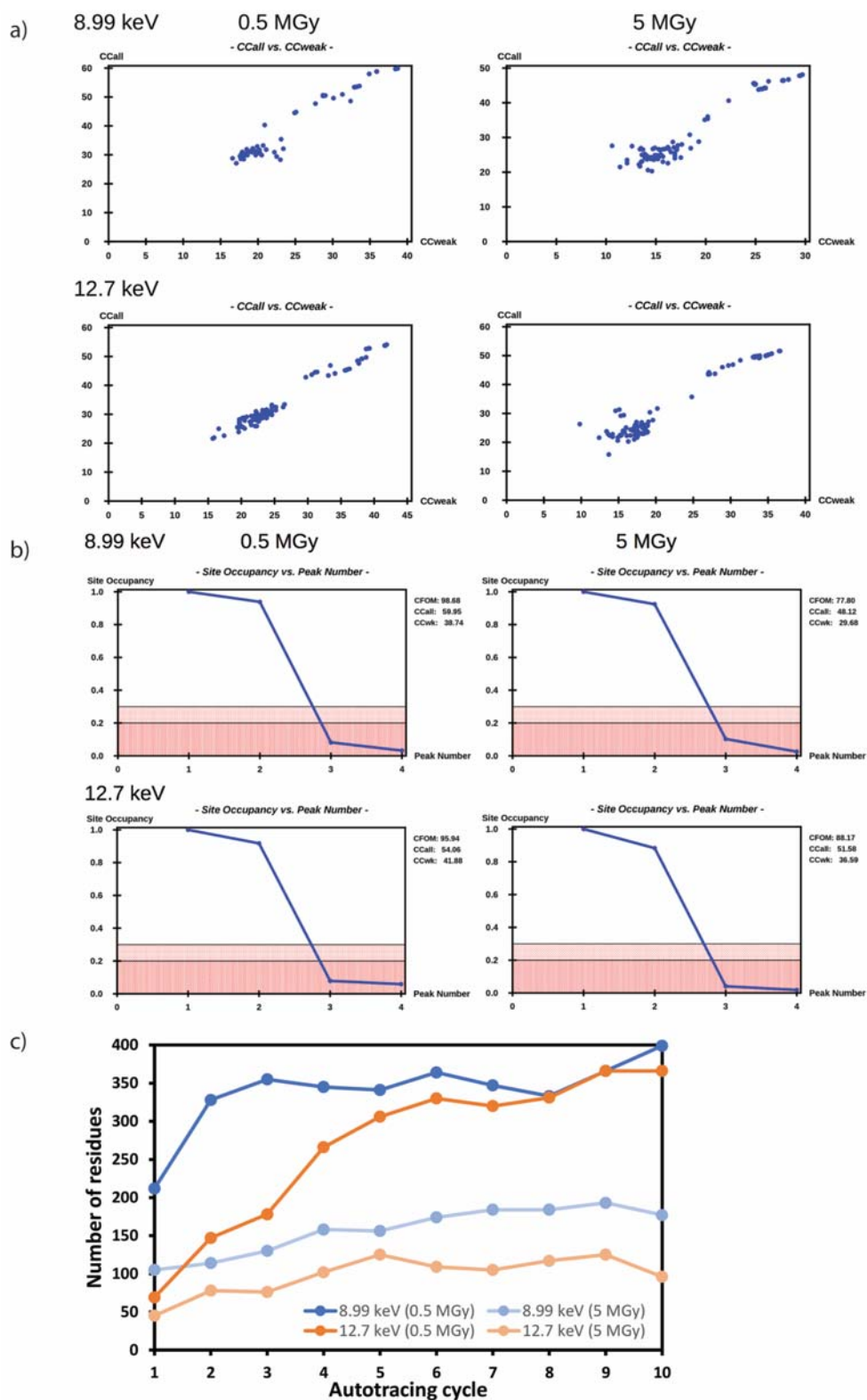
**Figure S3** The effects of phase error improvement after LLG completion of residual sulfur atoms, maps without density modification (*Phaser*) or after density modification (*Phaser + parrot*) are contoured at  $1\sigma$  a) Surrounding residue Y<sup>92</sup>, b) surrounding the GFP chromophore CRO.



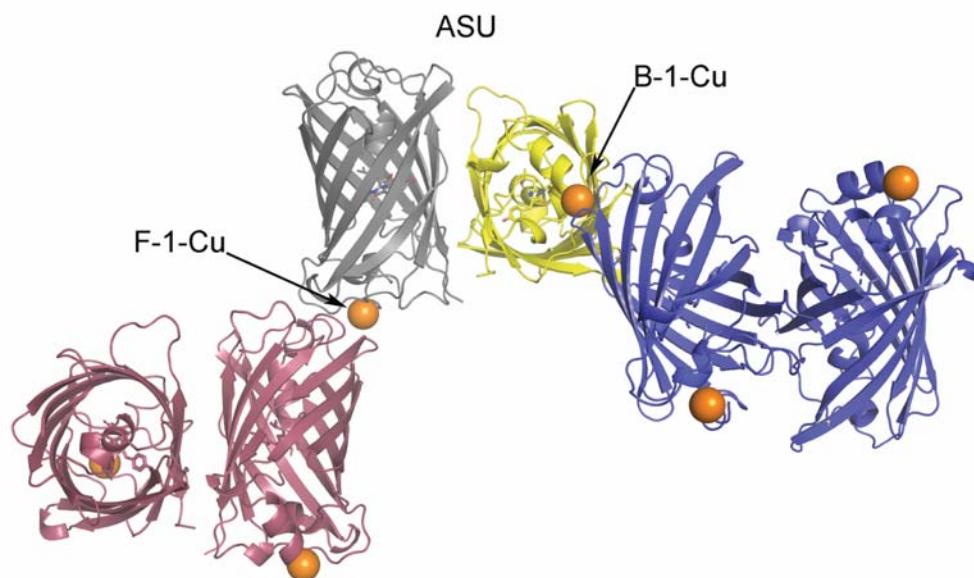
**Figure S4** X-ray fluorescence scan for the GHK-GFP crystals around the copper absorption edge (between 8957 and 9010 eV). The experimental anomalous scattering for Cu is 4.44  $\epsilon'$  at 8999.4 eV (1.3777 Å).



**Figure S5** Global radiation damage as a function of resolution for the dose series acquired at 8.99 keV (blue) and 12.7 keV (orange). a) Decrease of the diffraction limit with increasing dose (cutoff  $I/\sigma=1.0$ ). b) Decrease of the anomalous signal with increasing dose (cutoff  $CC_{1/2} \text{anom}=30\%$ ).

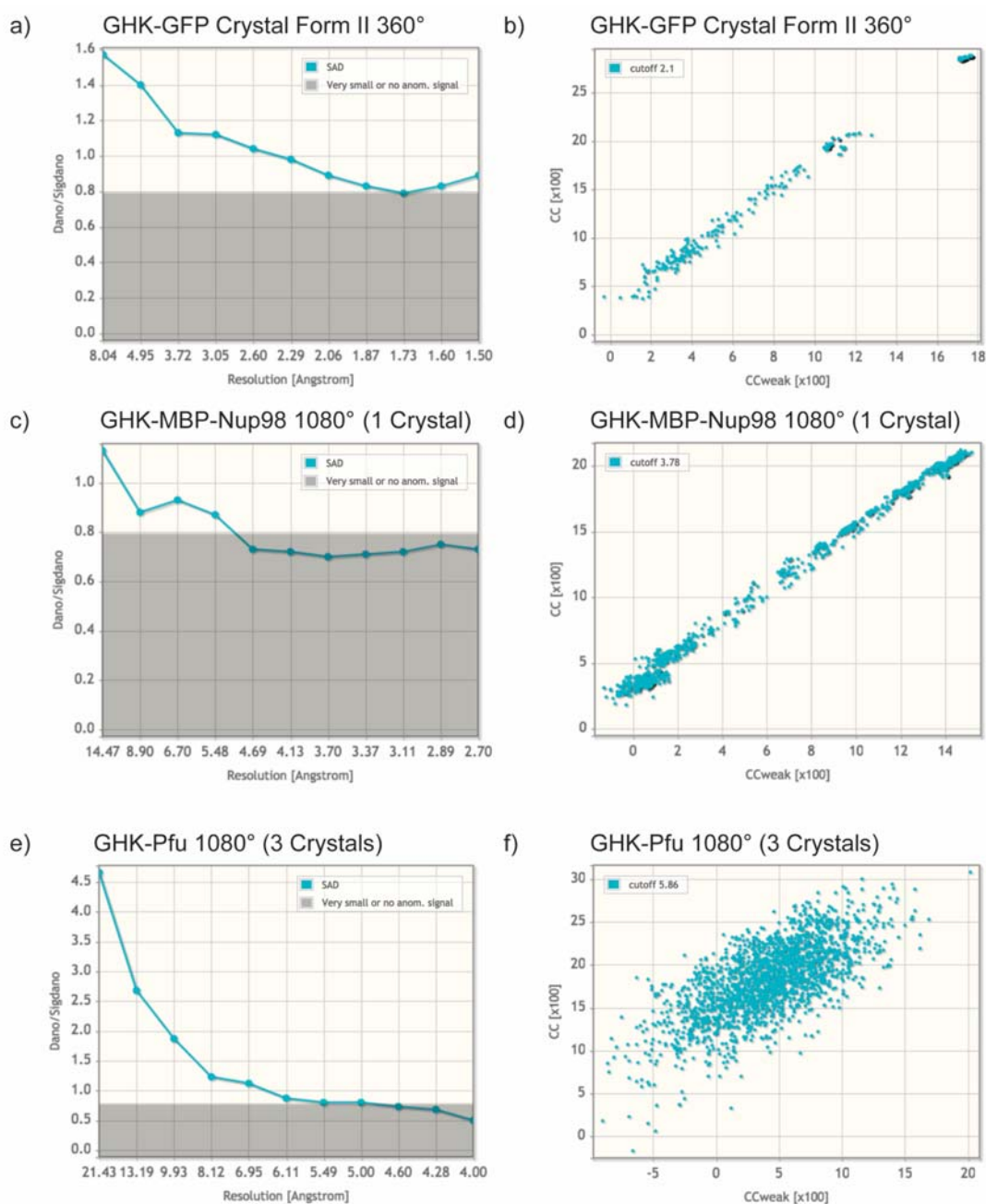


**Figure S6** Influence of radiation damage on substructure determination and autotracing by *SHELXC/D/E*. a) *SHELXD* correlation coefficients for substructure determination of the first (0.5 MGy) and last (5 MGy) datasets of the dose series. c) Autotracing of the 0.5 MGy and 5 MGy datasets (acquired at the Cu absorption edge and at 12.7 keV) by *SHELXE*.

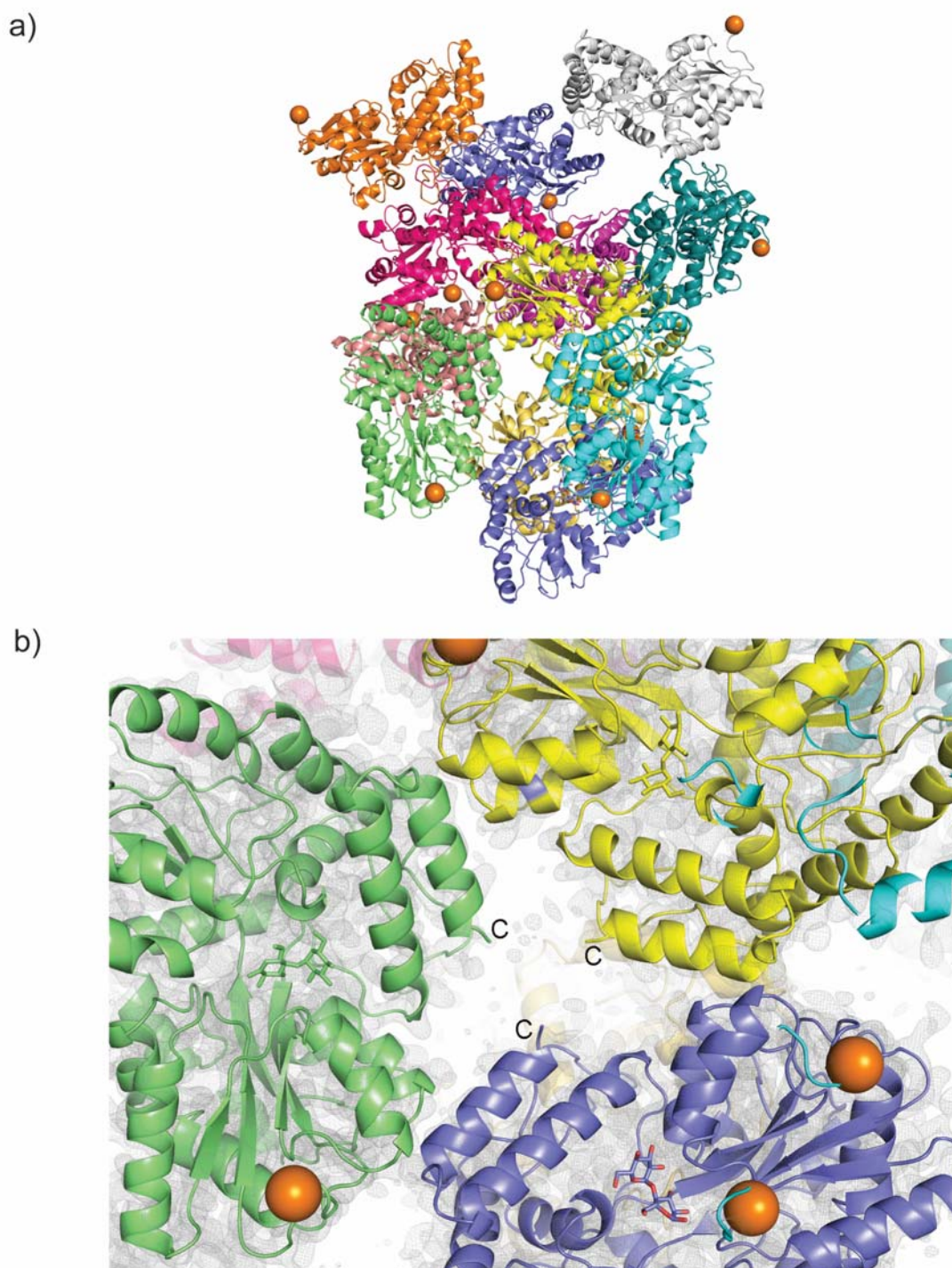


**Figure S7** Copper binding sites between different GHK-GFP molecules within the ASU. The ASU dimer is coloured grey and yellow and symmetry related molecules coloured raspberry and blue.

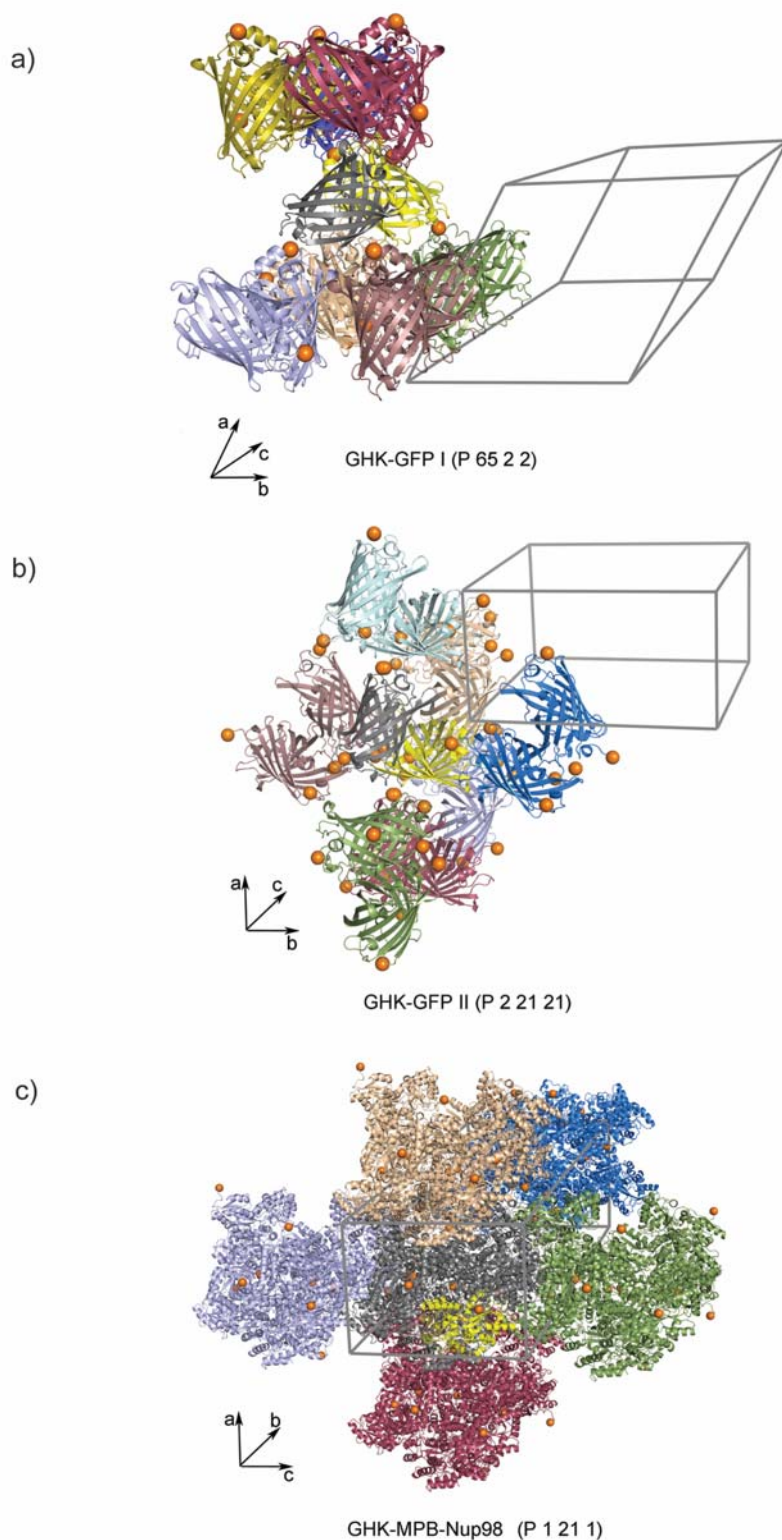




**Figure S8** Output from *CRANK2* pipeline run via *CCP4* online (<https://ccp4serv7.rc-harwell.ac.uk/ccp4online>) for GHK-GFP crystal form II, GHK-MBP-Nup98 and GHK-Pfu. Panels a,c,e; Anomalous signal estimation output from *SHELXC*. Panels b,d,f; Profiles of *SHELXD* correlation coefficients (CC) for substructure determination. Results are shown for the datasets given in Table 1



**Figure S9** The ASU of GHK-MPB-Nup98 crystals is shown in a) as ribbons with each of the 12 molecules coloured differently, bound copper ions are shown as orange spheres. A close up of the C-terminal region from 3 molecules is shown in b) with the corresponding 2Fo-Fc map contoured at  $1\sigma$ . It was not possible to trace the chain any further due to the flexibility of the Nup98 (1-29) sequence.



**Figure S10** The crystal structures of a) GHK-GFP crystal form I, b) GHK-GFP crystal form II and c) GHK-MBP-Nup98. In each case the ASU is coloured grey with 1 molecule coloured yellow, symmetry related ASU are shown in different colours and the unit cell is drawn. Bound copper ions are shown as orange spheres.

**Table S1** Oligonucleotides sequences used

Oligo	Sequence (Vector Gibson assembly sites are in bold, GHK underlined)
GHK-GFP_F	5' <u>tcagactggtggcggccataaa</u> cgtaggagaacaactgttcaccggggtg
GHK-GFP_R	5' <b>gtcgagcctacgag</b> ctacgttatgccagcagcagtaacaaattccagtaggac
GHK-MBP-Nup98_F	5' <u>tcagactggtggcggccataaaaa</u> attgaagaaggtaaactggtaactctggattaacggcgat
GHK-MBP-Nup98_R	5' <b>gtcgagcctacgag</b> ctactgctgctgacctgaccgaacagaccacc
GHK-Pfu_F	5' <u>tcagactggtggcggccataaaa</u> tgattttagatgtggattacataactgaagaagga
GHK-Pfu_R	5' <b>gtcgagcctacgag</b> ctagtatctgaggtctcctttctgtatccaaatcc