Supplementary Information:

Hydroxymethylation of Cys121 by Formaldehyde

An unusual feature of the protein structure is a chemical modification of Cys 121, distant from the active site of the enzyme. During co-crystallization of $BiGF_2$•CBR1, the presence of excess formaldehyde, not sequestered by GSH, allowed the hydroxymethylation of S^{γ} of Cys121 as evidenced by positive difference density fitting a covalently bound hydroxymethyl moiety. The hydroxymethylcysteine is positioned in a cleft bounded by 3 alpha helices and parallel ß-sheets D, E and F of the Rossman fold. O^{ε} of the modified residue lies in a position occupied by water in our previous structure (1WMA), and is positioned 2.8Å from His207 $N^{\delta}1$. His207 $N^{\varepsilon}2$ is in turn hydrogen bound to Glu164 $O^{\varepsilon}2$ (2.8A). No other residues in CBR1 showed evidence of modification.

HMGSH is rapidly metabolized to formate by the glutathione-dependant formaldehyde dehydrogenases, while HMGSH remains reactive and can lead to additional modifications within the proteome. In this structure, we identified a hydroxymethylated cysteine. This modification has provided the first structural characterization of formaldehyde-modified cysteine in the PDB. Aldehydes react with residues including Cys and Lys resulting in modulation of protein function; although the importance of this process is commonly recognized no structural information with regard to Cys modification by formaldehyde has previously been available.

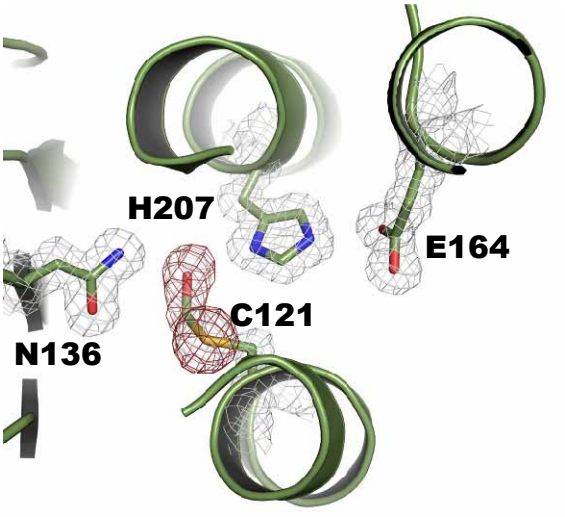

Figure S1: Environment and electron density surrounding hydroxymethylcysteine 121. The $2F_o$ - F_c map is contoured in gray at a level of 1 σ. The F_o - F_c map is contoured in red at a level of 2.75 σ.

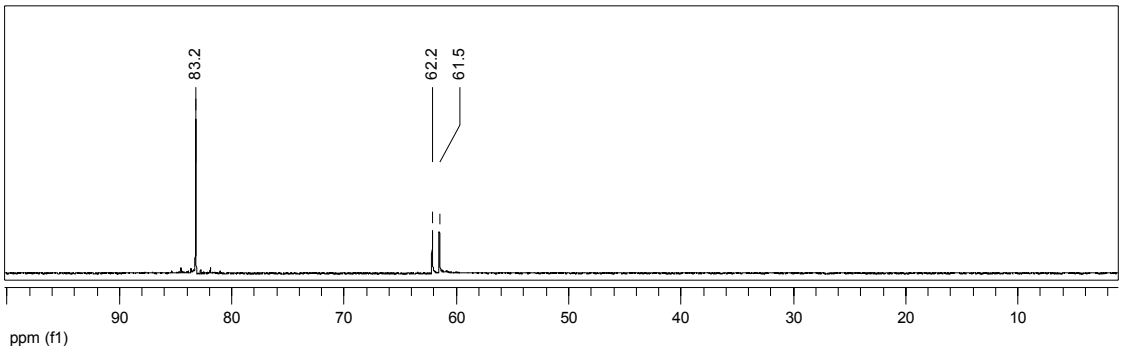

Figure S2: ^{13}C NMR spectrum (pH 8.5) of a solution of Glutathione and ^{13}C Formaldehyde 8:1 (details present in Materials and Methods).

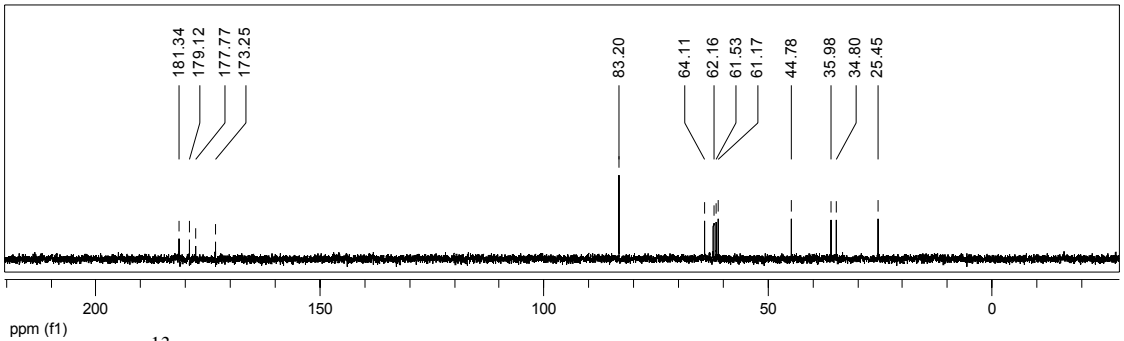

Figure S3: ^{13}C NMR (100.47 MHz) spectrum (pH 8.5) of a solution of Glutathione and Formaldehyde 8:1 (details present in Materials and Methods).

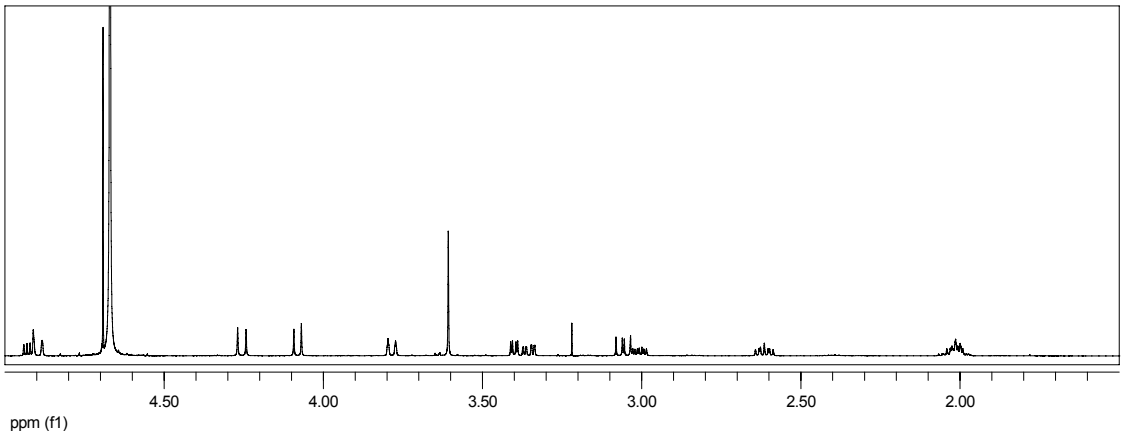

Figure S4: ^{1}H NMR (600 MHz) spectrum (pH 8.5) of a solution of Glutathione and Formaldehyde 8:1 (details present in Materials and Methods).

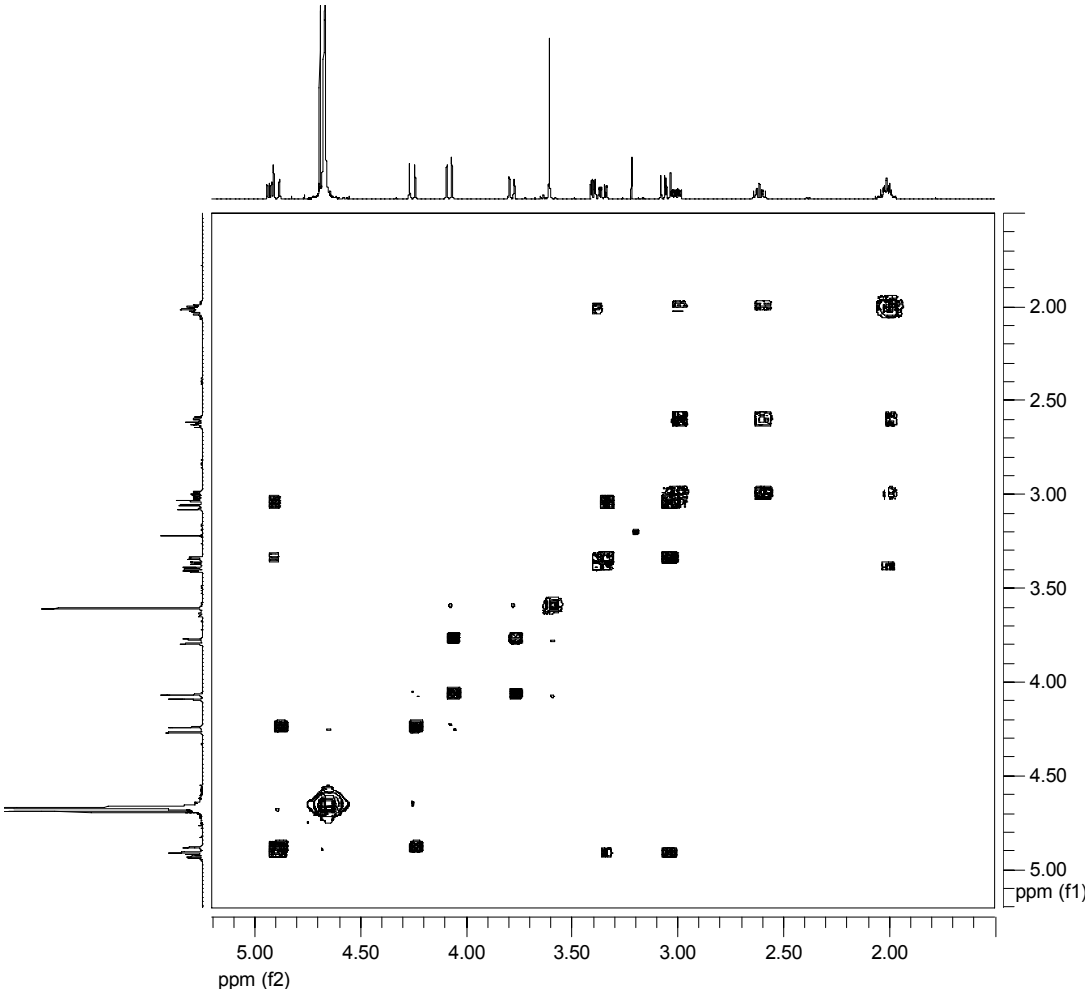

Figure S5: ^{1}H gCOSY NMR (599.67 MHz) spectrum of a solution of Glutathione and Formaldehyde 8:1 (details present in Materials and Methods).

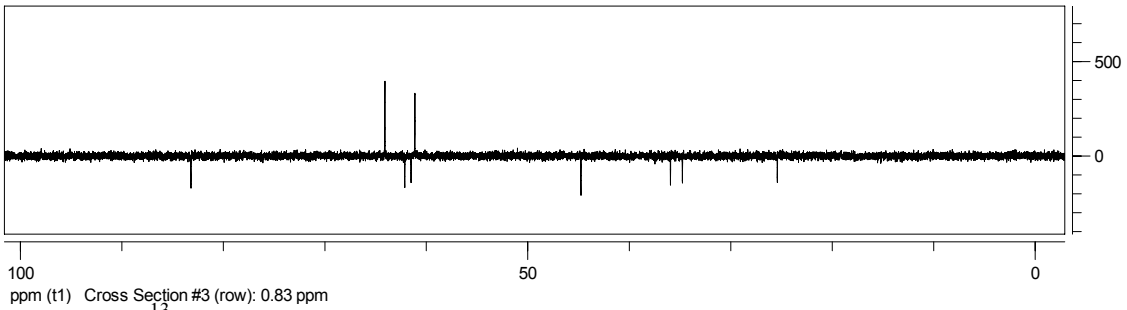

Figure S6: ^{13}C DEPT-135 (100.47 MHz) spectrum (pH 8.5) of a solution of Glutathione and Formaldehyde 8:1 (details present in Materials and Methods).