

**Supporting Information For**

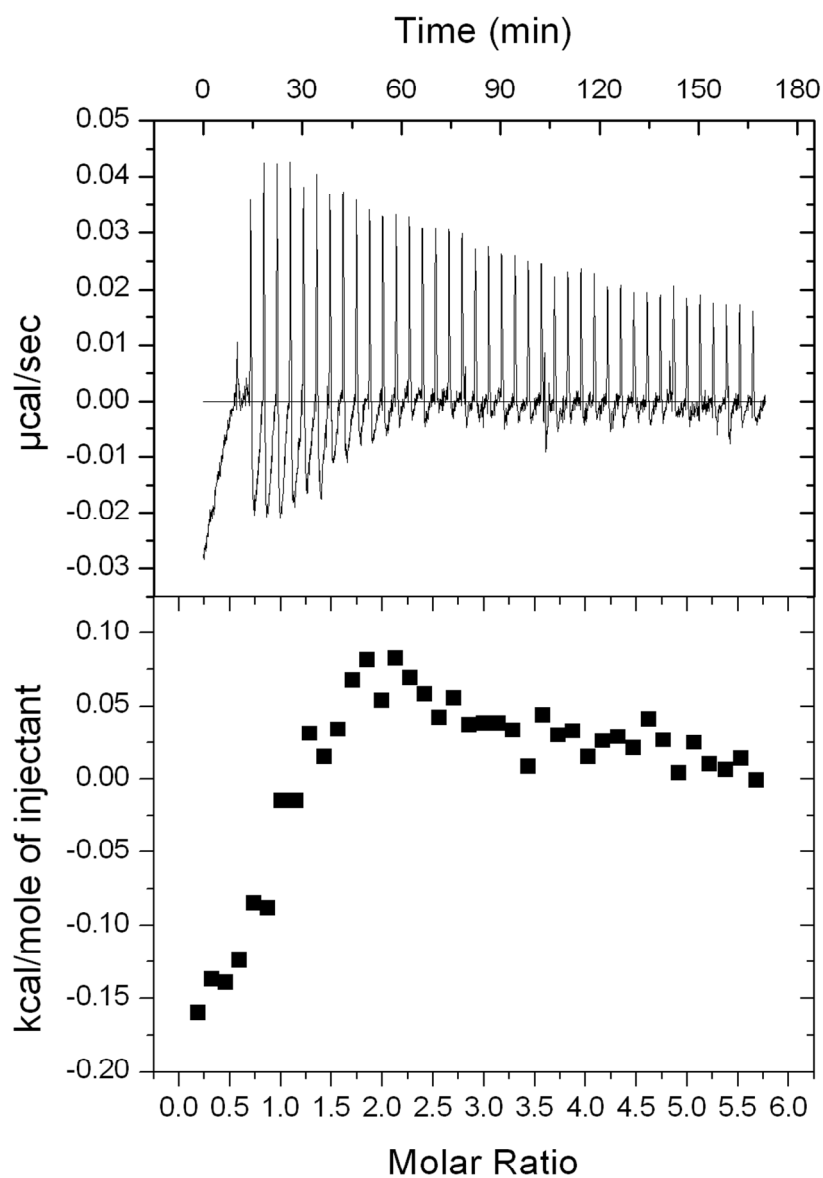
**Structural Characterization of Ferrous Ion Binding to Retinal Guanylate Cyclase Activator**

**Protein-5 from Zebrafish Photoreceptors.**

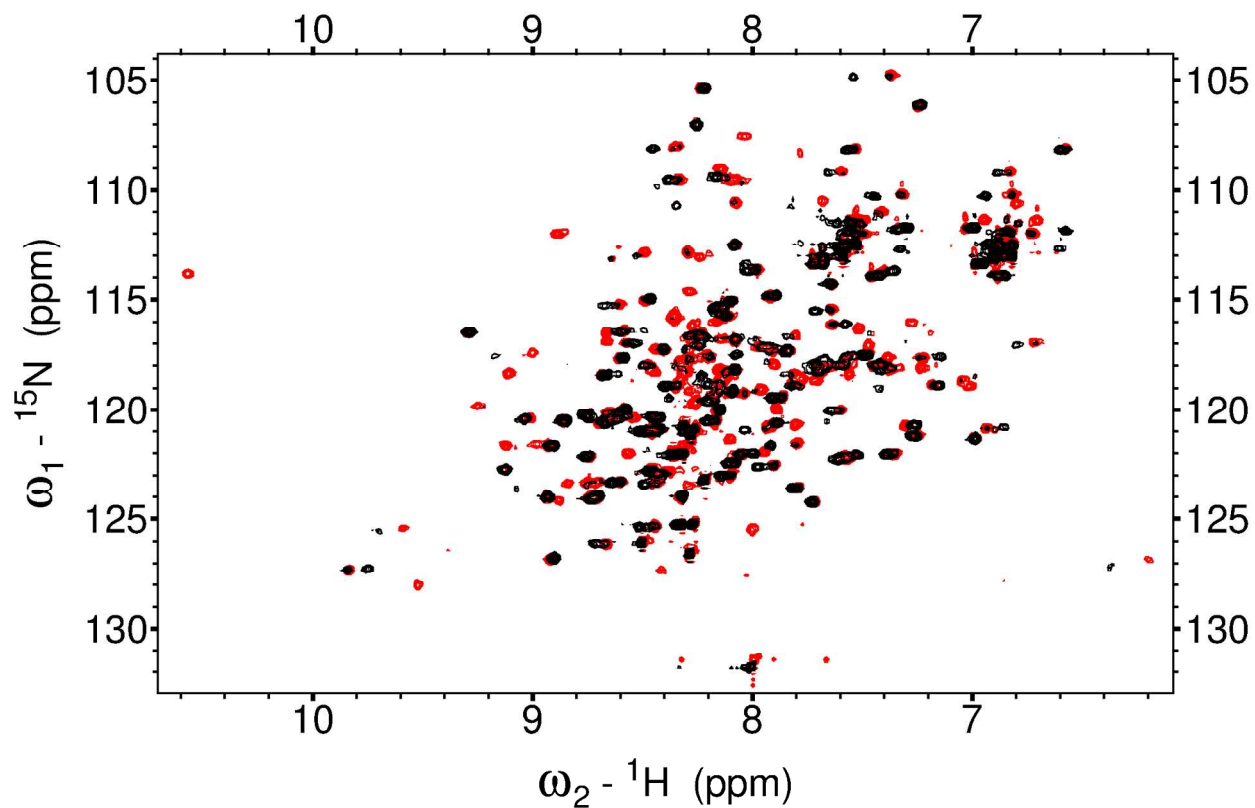
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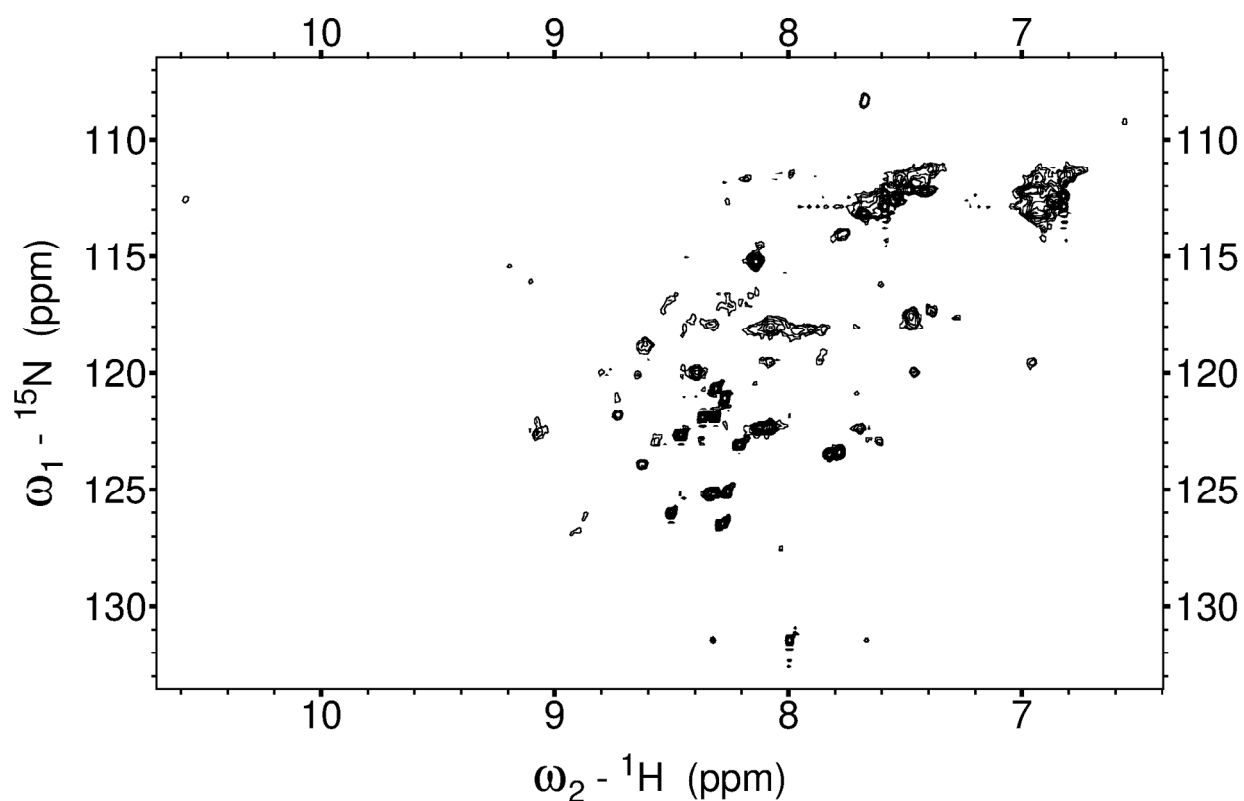
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**Figure S1.** ITC titration of GCAP5<sup>C15A/C17A</sup> with Fe<sup>2+</sup> ions in the presence of saturating Ca<sup>2+</sup> concentration (5 mM CaCl<sub>2</sub>) using the same experimental parameters as reported in the text except for the presence of 5 mM CaCl<sub>2</sub>. The binding isotherm in the bottom panel was fit to a one site model with  $\Delta H$  equal to -0.17 kcal/mol.



**Figure S2.** Overlay of  ${}^1\text{H}$ - ${}^{15}\text{N}$  HSQC spectra of  $\text{Mg}^{2+}$ -bound GCAP5 in the  $\text{Fe}^{2+}$ -free (red) and  $\text{Fe}^{2+}$ -bound (black) states. The spectra were recorded using the same experimental parameters as reported in the text except that 5 mM  $\text{MgCl}_2$  was present in both samples.



**Figure S3.** Two dimensional  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $\text{Ca}^{2+}$  bound GCAP5<sup>WT</sup>. The spectrum was recorded under the same conditions reported in the text for  $\text{Ca}^{2+}$ -free GCAP5 except that 5 mM  $\text{CaCl}_2$  was added to the sample. Residues in unstructured loop regions give rise to sharp peaks near the middle of the spectrum, because of segmental motions in these unstructured regions. The resonances assigned to residues in the structured regions are significantly broadened due to  $\text{Ca}^{2+}$ -induced protein aggregation.