

Supporting Information For

**Structural Characterization of Ferrous Ion Binding to Retinal Guanylate Cyclase Activator
Protein-5 from Zebrafish Photoreceptors.**

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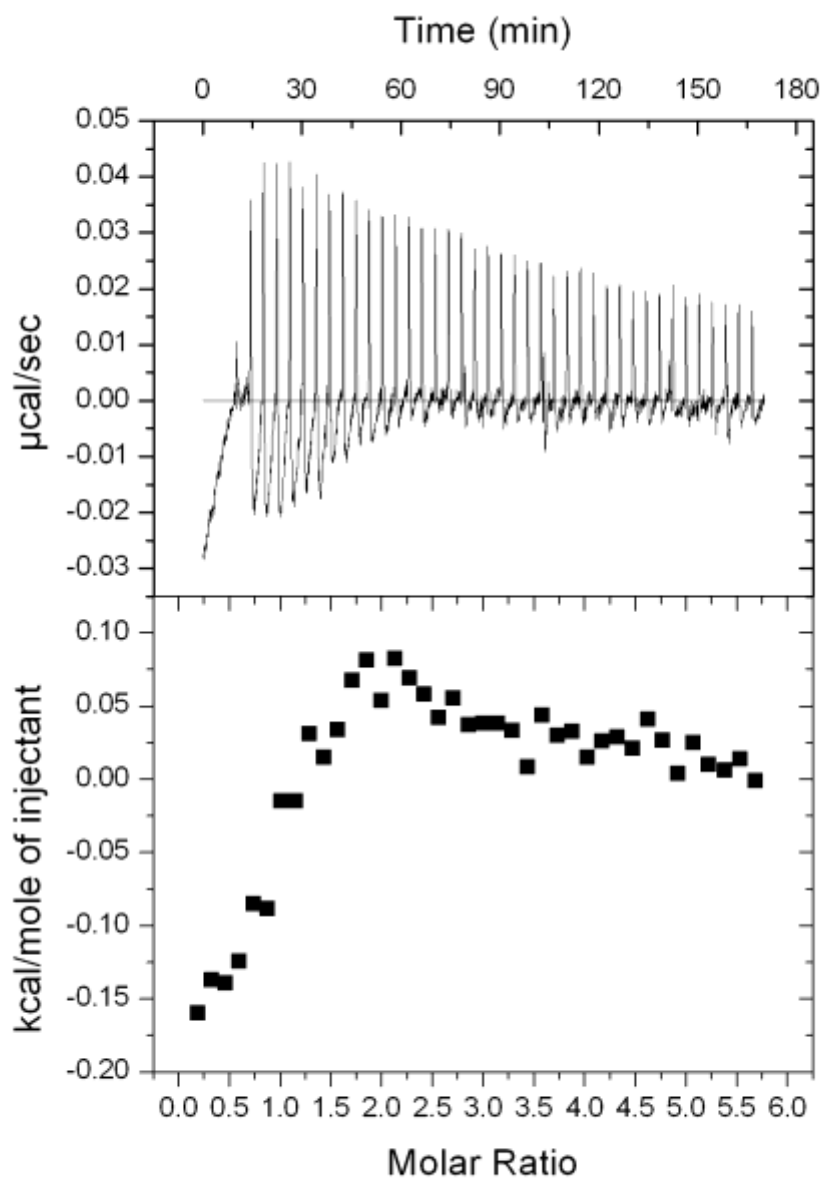


Figure S1. ITC titration of GCAP5^{C15A/C17A} with Fe²⁺ ions in the presence of saturating Ca²⁺ concentration (5 mM CaCl₂) using the same experimental parameters as reported in the text except for the presence of 5 mM CaCl₂. The binding isotherm in the bottom panel was fit to a one site model with ΔH equal to -0.17 kcal/mol.

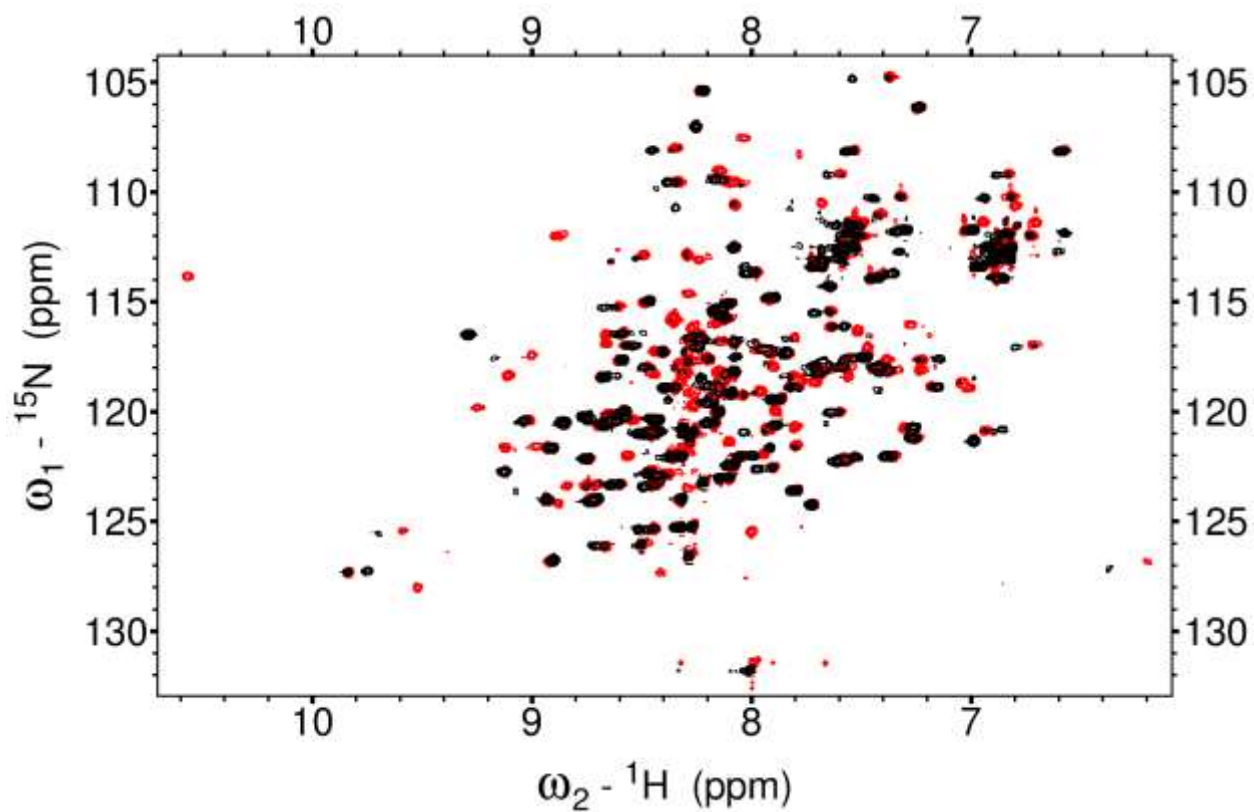


Figure S2. Overlay of ^1H - ^{15}N HSQC spectra of Mg^{2+} -bound GCAP5 in the Fe^{2+} -free (red) and Fe^{2+} -bound (black) states. The spectra were recorded using the same experimental parameters as reported in the text except that 5 mM MgCl_2 was present in both samples.

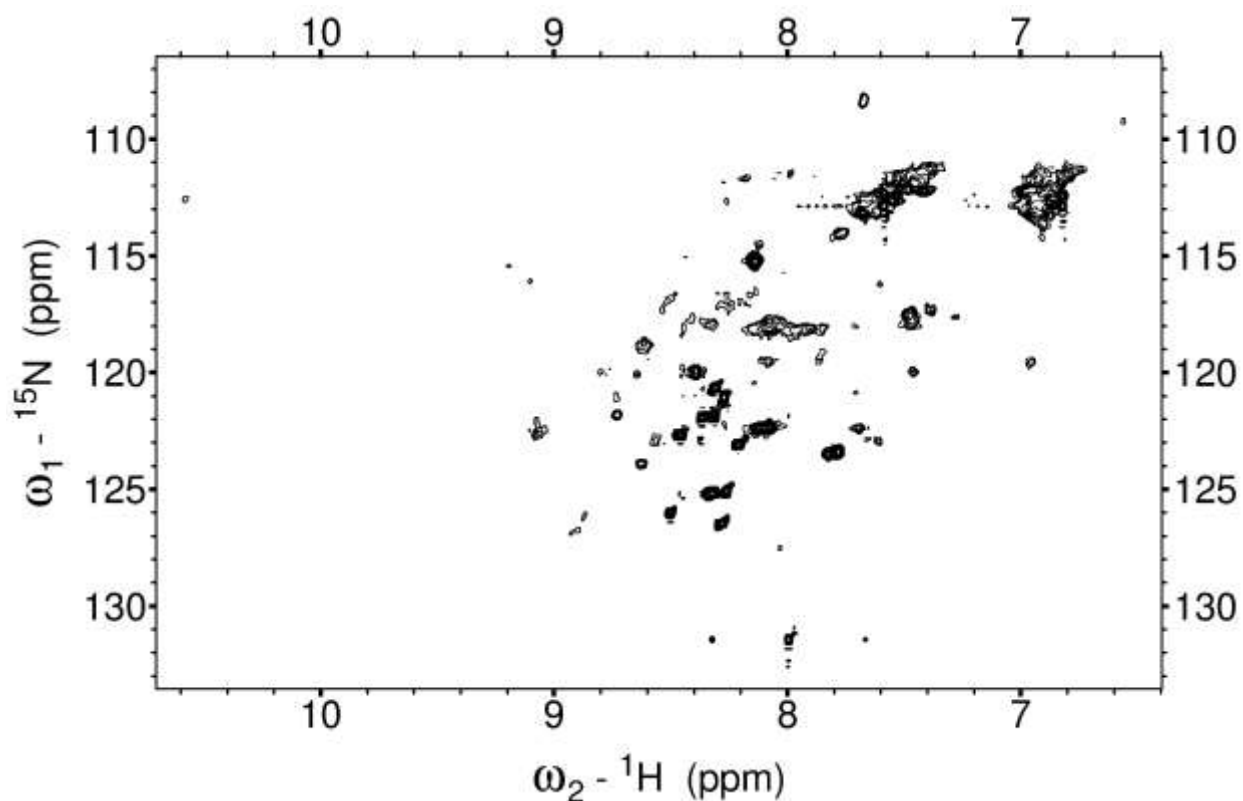


Figure S3. Two dimensional ^1H - ^{15}N HSQC spectra of Ca^{2+} bound GCAP5^{WT}. The spectrum was recorded under the same conditions reported in the text for Ca^{2+} -free GCAP5 except that 5 mM CaCl_2 was added to the sample. Residues in unstructured loop regions give rise to sharp peaks near the middle 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 Ca^{2+} -induced protein aggregation.