

Supporting Information

Efficient Localization of a Native Metal Ion Within a Protein by Cu^{2+} -Based EPR Distance Measurements

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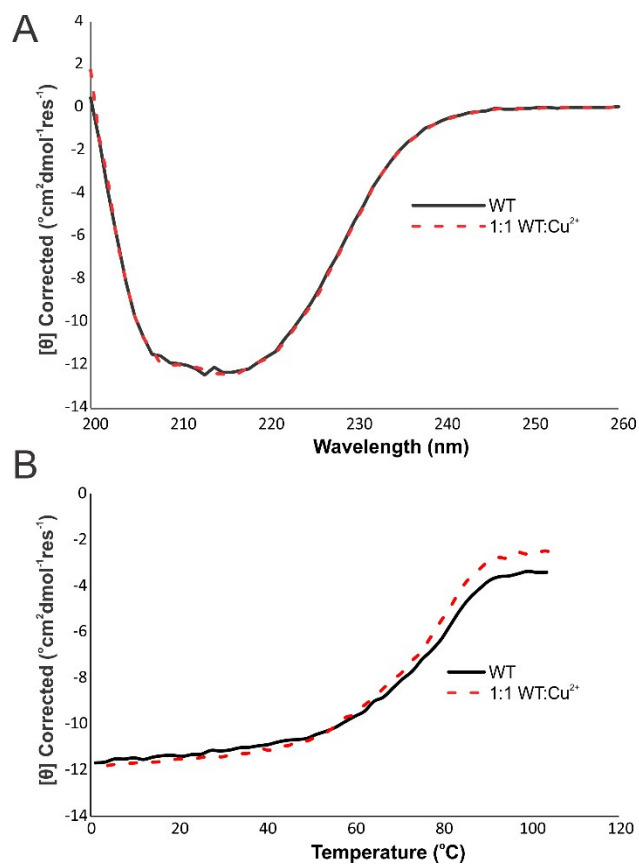


Figure S1. A) Circular dichroism (CD) spectra of WT GB1 with (red dashed) and without (black) 1 equivalent of Cu^{2+} . B) Temperature melts of WT GB1 with and without 1 equivalent of Cu^{2+} .

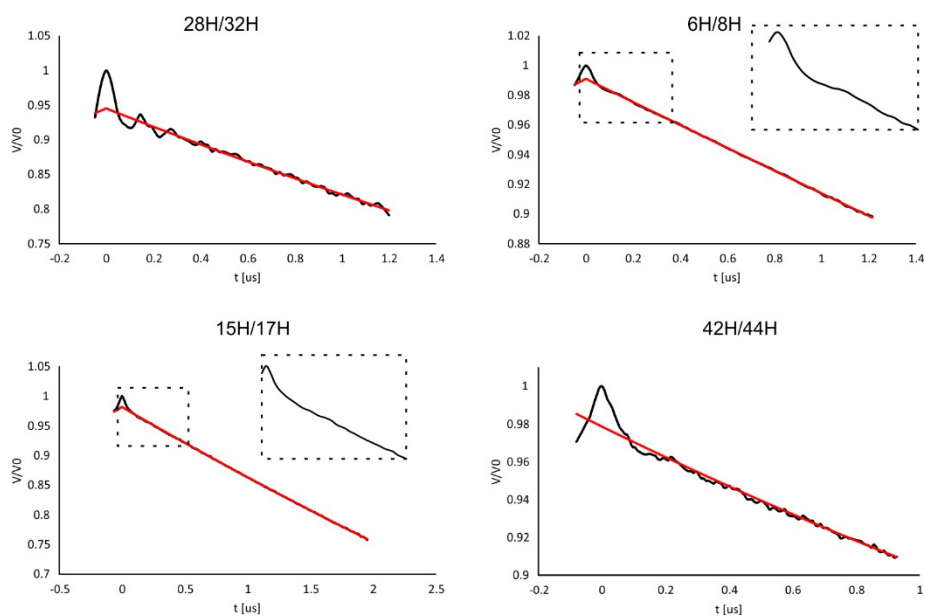


Figure S2. Raw time domain DEER data for the four GB1 mutants. Select insets are provided for a clearer view of the modulations.

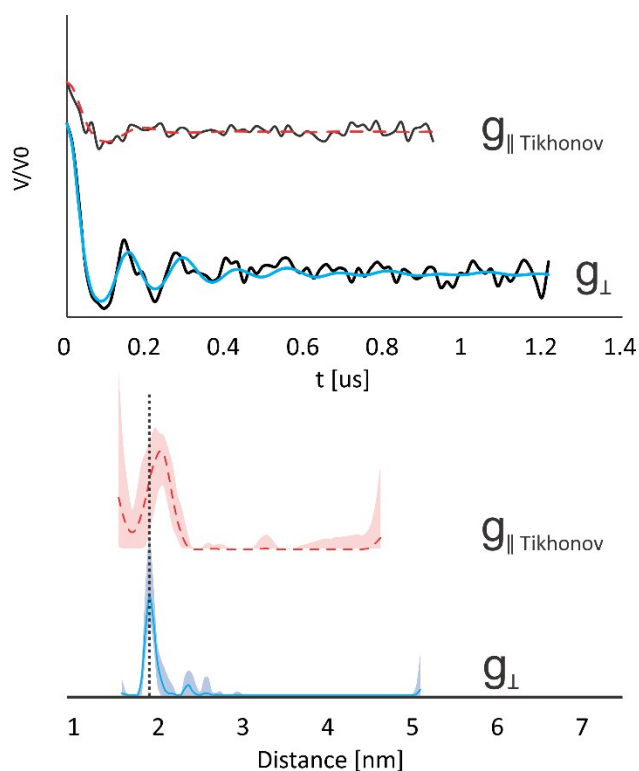


Figure S3. A) Time domain $g_{\parallel}$ DEER data on 28H/32H GB1 for analysis of orientational effects. Because of the poor modulation depth and signal to noise ratio obtained at $g_{\perp}$, the time domain data was filtered to remove high frequency noise and nuclear modulation effects (>10 MHz). This data is shown fit by Tikhonov regularization (red dashed). $g_{\parallel}$ data (blue) is shown for comparison. B) Distance distributions from the above DEER data. The shaded region indicates the uncertainty of the distribution. $g_{\parallel}$ analyses show agreement of the most probable distance within 0.1 nm. The distribution width is broader at $g_{\perp}$ than $g_{\parallel}$, likely due to the noise being on the same order of magnitude as the dampening dipolar modulations.

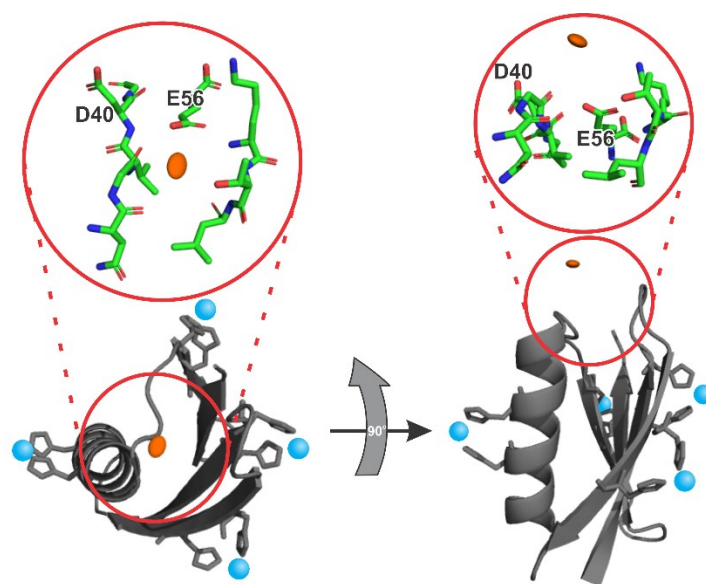


Figure S4. Trilateration of the WT GB1 native binding site performed using mtsslSuite⁸. The target site is located in proximity to the same residues of that found via MMM⁶⁻⁷.

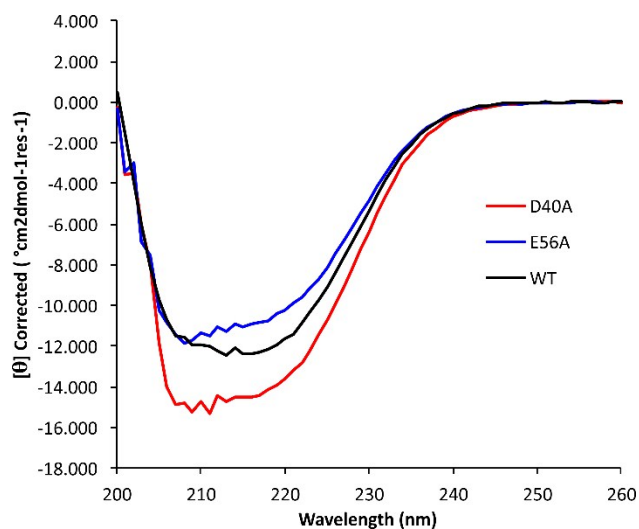


Figure S5. Circular Dichroism spectra of WT, D40A, and E56A GB1. The CD signature of the D40A and E56A mutants show some small differences from WT GB1. These subtle differences in the CD spectra may indicate minor structural variations, but these differences are not significant enough to account for the loss in Cu²⁺ binding that we observed.

REFERENCES

1. Cunningham, T. F.; Putterman, M. R.; Desai, A.; Horne, W. S.; Saxena, S., The Double Histidine Cu²⁺-Binding Motif: A Highly Rigid, Site-Specific Spin Probe for Electron Spin Resonance Distance Measurements. *Angew. Chem. Intl. Ed.* **2015**, *54*, 6330.
2. Stoll, S.; Schweiger, A., EasySpin, a Comprehensive Software Package for Spectral Simulation and Analysis in EPR. *J. Magn. Res.* **2006**, *178* (1), 42-55.
3. Pannier, M.; Veit, S.; Godt, A.; Jeschke, G.; Spiess, H. W., Dead-Time Free Measurement of Dipole-Dipole Interactions Between Electron Spins. *J. Magn. Res.* **2000**, *142*, 331-340.
4. Jeschke, G.; Chechik, V.; Ionita, P.; Godt, A.; Zimmermann, H.; Banham, J.; Timmel, C.; Hilger, D.; Jung, H., DeerAnalysis 2006 - a Comprehensive Software Package for Analyzing Pulsed ELDOR Data. *Appl. Magn. Reson.* **2006**, *30*, 473-498.
5. Bowen, A. M.; Jones, M. W.; Lovett, J. E.; Gaule, T. G.; McPherson, M. J.; Dilworth, J. R.; Timmel, C. R.; Harmer, J. R., Exploiting Orientation-Selective DEER: Determining Molecular Structure in Systems Containing Cu (II) Centres. *Phys. Chem. Chem. Phys.* **2016**, *18* (8), 5981-5994.
6. Jeschke, G., MMM: A Toolbox for Integrative Structure Modeling. *Protein Sci.* **2018**, *27* (1), 76-85.
7. Ghosh, S.; Saxena, S.; Jeschke, G., Rotamer Modelling of Cu(II) Spin Labels Based on the Double-Histidine Motif. *Appl. Magn. Reson.* **2018**, *49* (11), 1281-1298.
8. Hagelueken, G.; Abdullin, D.; Ward, R.; Schiemann, O., mtsslSuite: In Silico Spin Labelling, Trilateration and Distance-Constrained Rigid Body Docking in PyMOL. *Mol. Phys.* **2013**, *111* (18-19), 2757-2766.