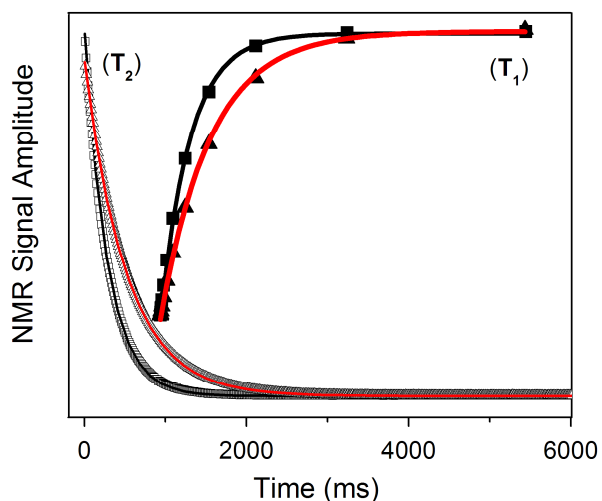


**THE ROLE OF CALCIUM IN METALLOENZYME: EFFECTS OF CALCIUM
REMOVAL ON THE AXIAL LIGATION GEOMETRY AND MAGNETIC
PROPERTIES OF THE CATALYTIC DIHEME CENTER IN MauG**

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Figure S1. The weight-normalized NMR signal amplitude of native (fitted with black traces) and Ca²⁺-depleted (fitted with red traces) MauG as a function of time. The relaxivity T_1 and T_2 values were obtained from the fitting of these data. The r_1 and r_2 values were calculated from the T_1 and T_2 values according to eqs. 2 and 3 as described in the text.



Further Details of the Proton NMR Relaxometry Study

According to the accepted modified equations of Solomon¹ and Bloembergen.²

$$\frac{1}{T_1} = \frac{[M]}{55.5} \frac{n}{T_{1m} + \tau_m} \quad (\text{S1})$$

$$\frac{1}{T_2} = \frac{[M]}{55.5} \frac{n}{T_{2m} + \tau_m} \quad (\text{S2})$$

Where n is the water molecules in the first coordination sphere of the paramagnetic ion of the molarity m , T_{1m} and T_{2m} are the longitudinal and the transverse relaxation times of bonded protons and τ_m is the residence time of bonded water. According to equation 1 and 2, the relaxivity of MauG could be influenced by the number of water molecular in the first coordination sphere of ion (n). The Ca^{2+} ion in MauG is surrounded by 4 water molecules. The removal of Ca^{2+} from MauG could potentially decrease number of water molecular in the first sphere of ion. When n decrease, both of longitudinal and transverse relaxivity of MauG decrease. Besides, the change of T_{1m} and T_{2m} also influences the relaxivity of MauG. The T_{1m} and T_{2m} can be determined by equation S3-S5.

$$\frac{1}{T_{1m}} = \frac{2}{15} C \left[\frac{7\tau_{c2}}{1 + \omega_s^2 \tau_{c2}^2} + \frac{3\tau_{c1}}{1 + \omega_l^2 \tau_{c1}^2} \right] + \frac{2}{3} \left(\frac{A}{\hbar} \right)^2 S(S+1) \frac{\tau_{e2}}{1 + \omega_s^2 \tau_{e2}^2} \quad (\text{S3})$$

$$\frac{1}{T_{2m}} = \frac{C}{15} \left[4\tau_{c1} + \frac{13\tau_{c2}}{1 + \omega_s^2 \tau_{c2}^2} + \frac{3\tau_{c1}}{1 + \omega_l^2 \tau_{c1}^2} \right] + \frac{1}{3} \left(\frac{A}{\hbar} \right)^2 S(S+1) \left(\tau_{e1} + \frac{\tau_{e2}}{1 + \omega_s^2 \tau_{e2}^2} \right) \quad (\text{S4})$$

$$C = \gamma_l^2 g_e^2 \mu_B^2 S(S+1) (r^{-6}) \quad (\text{S5})$$

Where ω_l and ω_s are the nuclear and electron larmor precession frequencies, respectively. S is the total electron spin. A/\hbar is the electron-nuclear hyperfine coupling constant. τ_c and τ_e are the correlation times for dipolar and spin exchange interactions respectively. γ_l is the nuclear gyromagnetic ratio. g_e is the spectroscopic splitting factor. μ_B is Bohr magneton, r is the proton-metal distance. EPR/ Mössbauer data indicate that when Ca^{2+} ion was removed from MauG, spin quantum number of high-spin heme decreased from 5/2 to 1/2. According to equation S3-S5, the decrease of S increases both T_{1m} and T_{2m} . As shown in equation S1 and S2, the increase of T_{1m} and T_{2m} causes decrease of the longitudinal and transverse relaxivity ($1/T_1$ and $1/T_2$), respectively.

References

- (1) Solomon, I. *Phys. Rev.* **1955**, 99.
- (2) Bloembergen, N. *J. Chem. Phys.* **1957**, 27.