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Supplementary Information:

The following chemical equations describe the sequential binding of two metals by an enzyme,

$$E + M \rightleftharpoons EM \; ; \; K_{A1}$$

$$EM + M \rightleftharpoons EMM \; ; \; K_{A2}$$

where *E*, *EM*, and *EMM* are defined as the concentration of apoR2, mononuclear $Mn^{II}R2$, and dinuclear $Mn_2^{II}R2$, respectively. The association constants (K_{A1} and K_{A2}) for each binding site can be described by the following equations.

$$K_{A1} = \frac{EM}{M_F \cdot E_F} \qquad (1)$$

$$K_{A2} = \frac{EMM}{M_F \cdot E_M} \qquad (2)$$

Additionally, two equations can be defined to describe the mass balance of each species during the titration of apoR2 with Mn^{II},

$$E_T = E_F + EM + EMM \qquad (3)$$

$$M_T = M_F + EM + 2EMM \qquad (4)$$

where E_T, E_F, M_T, and M_F are defined as the concentration of total enzyme present, free enzyme (apoR2), total Mn^{II} added, and unbound Mn^{II} (aquaMn^{II}), respectively. From equations (1) - (4), an algebraic expression can be derived for both E_F and M_F.

$$E_T = E_F + EM + EMM$$
$$E_T - E_F = EM + EMM = K_{A1} \cdot M_F \cdot E_F + K_{A1} \cdot K_{A2} \cdot E_F \cdot M_F^2$$
$$E_T = E_F + K_{A1} \cdot M_F \cdot E_F + K_{A1} \cdot K_{A2} \cdot E_F \cdot M_F^2 = E_F(1 + K_{A1} \cdot M_F + K_{A1} \cdot K_{A2} \cdot M_F^2)$$

$$E_F = \frac{E_T}{(1 + K_{A1} \cdot M_F + K_{A1} \cdot K_{A2} \cdot M_F^2)} \qquad (5)$$

$$M_T = M_F + EM + 2EMM$$
$$M_F = M_T - EM - 2EMM = M_T - EM - 2K_{A2} \cdot EM \cdot M_F$$
$$M_F + 2K_{A2} \cdot EM \cdot M_F = M_T - EM$$
$$M_F(2K_{A2} \cdot EM + 1) = M_T - EM$$

$$M_F = \frac{M_T - EM}{2K_{A2} \cdot EM + 1} \qquad (6)$$

Substitution of E_T and M_F into equation (1) and solving for EM yields,

$$EM = K_{A1} \cdot M_F \cdot E_F$$
$$EM = K_{A1} \cdot M_F \cdot \frac{E_T}{(1 + K_{A1} \cdot M_F + K_{A1} \cdot K_{A2} \cdot M_F^2)}$$
$$EM(1 + K_{A1} \cdot M_F + K_{A1} \cdot K_{A2} \cdot M_F^2) = K_{A1} \cdot M_F \cdot E_T$$
$$EM + K_{A1} \cdot EM \cdot \left(\frac{M_T - EM}{2K_{A2} \cdot EM + 1}\right) + K_{A1} \cdot K_{A2} \cdot EM \cdot \left(\frac{M_T - EM}{2K_{A2} \cdot EM + 1}\right)^2 = K_{A1} \cdot E_T \cdot \left(\frac{M_T - EM}{2K_{A2} \cdot EM + 1}\right)$$

$$(2K_{A2} \cdot EM + 1)^2 \cdot EM + K_{A1} \cdot EM(M_T - EM)(2K_{A2} \cdot EM + 1) + K_{A1} \cdot K_{A2} \cdot EM(M_T - EM)^2$$
$$= K_{A1} \cdot E_T(M_T - EM)(2K_{A2} \cdot EM + 1)$$

followed by the expansion of the polynomial results in the following expression,

$$EM(4K_{A2}{}^2 \cdot EM^2 + 4K_{A2} \cdot EM + 1) + K_{A1} \cdot EM(2K_{A2} \cdot M_T \cdot EM + M_T - 2K_{A2} \cdot EM^2 - EM)$$
$$+K_{A1} \cdot K_{A2} \cdot EM(M_T{}^2 - 2M_T \cdot EM + EM^2) = K_{A1} \cdot E_T(2K_{A2} \cdot M_T \cdot EM + M_T - 2K_{A2} \cdot EM^2 - EM)$$

which simplifies to the final form, shown below.

$$(4K_{A2}{}^2 - K_{A1} \cdot K_{A2})EM^3 + (4K_{A2}{}^2 + 2K_{A1} \cdot K_{A2} \cdot E_T - K_{A1})EM^2$$
$$+(1 + K_{A1} \cdot M_T + K_{A1} \cdot E_T + K_{A1} \cdot K_{A2} \cdot M_T{}^2 - 2K_{A1} \cdot K_{A2} \cdot M_T \cdot E_T)EM \qquad (7)$$
$$-K_{A1} \cdot E_T \cdot M_T = 0$$

The roots of the final cubic equation can be numerically solved, where each of the above terms are simplified as follows:

$$A(EM)^3 + B(EM)^2 + C(EM) + D = 0 \qquad (8)$$

where *A*, *B*, *C*, and *D* are defined as:

$$A = 4K_{A2}^{\ 2} - K_{A1} \cdot K_{A2}$$
$$B = 4K_{A2}^{\ 2} + 2K_{A1} \cdot K_{A2} \cdot E_T - K_{A1}$$
$$C = 1 + K_{A1} \cdot M_T + K_{A1} \cdot E_T + K_{A1} \cdot K_{A2} \cdot M_T^{\ 2} - 2K_{A1} \cdot K_{A2} \cdot M_T \cdot E_T$$
$$D = -K_{A1} \cdot E_T \cdot M_T$$

Once a numerical solution is obtained for *EM*, substitution into (2) and solving for *EMM* gives the following expression:

$$EMM = K_{A2} \cdot M_F \cdot EM$$

$$EMM = K_{A2} \cdot EM \cdot \frac{M_T - EM}{2K_{A2} \cdot EM + 1} \qquad (9)$$

Thus, the concentration of all EPR observable species M_F, *EM*, and *EMM* are expressed in terms of K_{A1}, K_{A2}, M_T, and E_T, where M_T and E_T are known quantities. The values of K_{A1} and K_{A2} are then varied to obtain the best fit to the experimental data.

Figure S1. Perpendicular and parallel mode X-band EPR spectra for 3 eq. Mn^{II} added to apoR2 in the presence of 100 mM KCl (dashed line) and absence of any KCl (solid line). Both samples contain approximately 1 mM apoR2 in 25mM Hepes, 5% glycerol, pH 7.6. In the absence of KCl, all of the added Mn^{II} observed as the standard 6-line signal at g = 2.0 indicative of aquaMn^{II}. In the presence of 100 mM KCl 1 eq. of aquoMn^{II} is observed and 1 eq. of Mn_2^{II}R2 species present. Instrumental conditions: microwave frequency, 9.62 GHz ($B_1 \perp B$), 9.26 GHz ($B_1 \parallel B$); microwave power, 0.2 mW ($B_1 \perp B$), 2.0 mW ($B_1 \parallel B$); temperature, 12 K.

Figure S2. Equivalents of observed paramagnetic species as a function of added Mn^{II} (20% glycerol), and theoretical curves for a two site sequential binding model with $K_1 \approx 1.0 \times 10^5\ M^{-1}$ and $K_2 \approx 1.4 \times 10^4\ M^{-1}$. The species are Mn^{II}R2 ( ), Mn_2^{II}R2 ( ), and aquaMn^{II} ( ). Buffer conditions 100 mM Tris, 20% glycerol, pH 7.5. Under these conditions, 3.4 equivalents of Mn^{II} were observed to bind within apoR2.

Figure S1

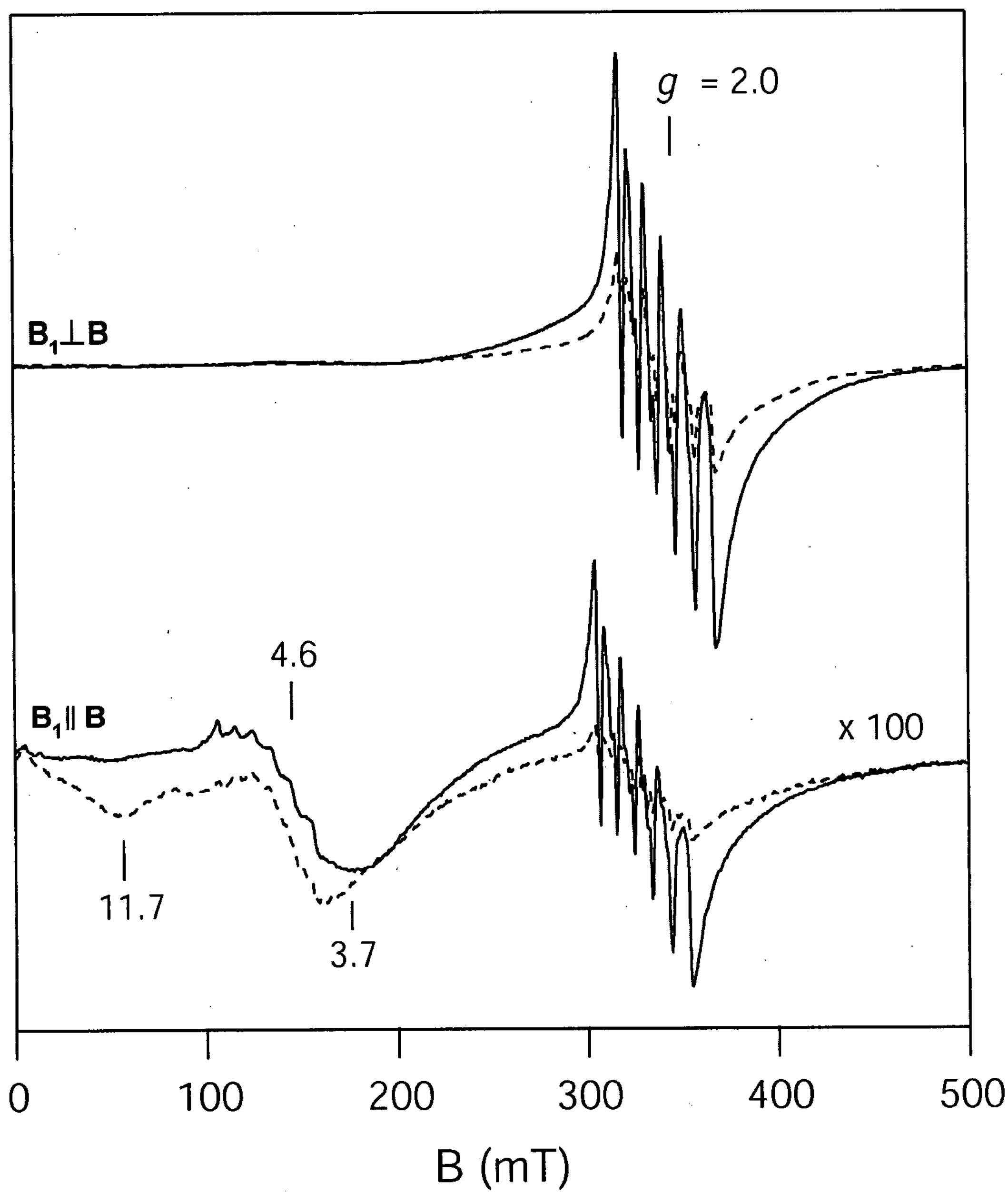

Figure S2