Supporting Information

The active form of *Chlamydia trachomatis* ribonucleotide reductase R2 protein contains a heterodinuclear Mn(IV)/Fe(III) cluster with *S* = 1 ground state

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Preparation of Mn^{IV}/Fe^{III}-R2 for Mössbauer Analysis: The heterobinuclear nature of the *Ct* RNR cofactor raises technical issues not previously encountered in investigations of conventional class I RNRs with their homobinuclear (diiron) cofactors. The challenge, in general, is to ensure that each metal occupies only its designated site (assuming that only one of two possible heterobinuclear forms is functional). As the requirement for Mn was only recently discovered,[1] development of a procedure to surmount this challenge and optimize the yield of the active protein is still in progress, but the following information is currently available. First, although precise quantitation is still in progress, it is already clear that, like other R2 proteins, *Ct* R2 takes up less than its theoretical complement of metal ions (one Mn and one Fe per β protein). As a result, a metal:β ratio of unity for either metal is actually a slight excess over the experimental reaction stoichiometry. Second, formation of the $Mn_2^{II/II}$ complex occurs (as evidenced by the complex EPR signal, which indicates the presence of coupled Mn^{II} ions),[2] but this complex is labile and unreactive to O_2. As a result, use of excess Mn^{II} does not prevent formation of the active Mn/Fe cofactor. By contrast, the $Fe_2^{II/II}$-R2 complex forms and is quite reactive to O_2, resulting in production of an $Fe_2^{III/III}$-R2 product that is presumably identical to the form that was characterized crystallographically.[3] Indeed, because Mössbauer probes only the Fe site, use of an excess of Mn (with respect to both the protein and the Fe) is actually desired because it disfavors competing formation of $Fe_2^{II/II}$-R2 reactant and $Fe_2^{III/III}$-R2 product. In fact, our current best procedure involves prior addition of 1.5 Mn per β (rather than the 1.0 ratio employed in this study) and slow addition of Fe^{II} (up to 0.75 equiv per β) into this solution in the presence of O_2. Dialysis against a chelator to remove unincorporated metal then results in a sample with < 5% of the "contaminating" $Fe_2^{III/III}$-R2 product. The sample used in the present study was prepared prior to development of this slightly better procedure, and it therefore had more of the contaminant (~ 25%). This contaminant proved not to be a serious detriment for the analysis, because its contribution could effectively be removed by subtraction.

As noted in Scheme 1, activation of *Ct* R2 by reaction of Mn^{II}/Fe^{II}-R2 with O_2 requires an "extra" electron. This situation is also operant in activation of conventional class I RNRs and has been investigated and discussed extensively.[4-6] At this point, the source and mechanism of transfer of the electron in the *Ct* R2 reaction are still under investigation. No reductant other than the divalent metal ions was added.

Spin Hamiltonian: Mössbauer spectra were analyzed with the spin Hamiltonian (equation 1), in which the first term represents the electron Zeeman effect, the second and third terms represent the axial and rhombic zero field splitting of the electronic ground state, the fourth term represents the interaction between the electric field gradient and the nuclear quadrupole moment of the Fe sites, the fifth term represents the magnetic hyperfine interaction of the electronic spin with the ^{57}Fe nucleus, and the last term represents the nuclear Zeeman interaction. The isomer shift is given relative to the centroid of αFe at room temperature. Simulations were carried out with respect to the total spin of the ground state, $S_{\mathrm{Total}} = 1$. The **A**-tensor in Equation 1 is given with respect to the total spin. Its relation to the intrinsic **a**-tensor of the Fe site is explained in the main manuscript. All tensors are assumed to be collinear.

$$\begin{aligned}\mathbf{H} = \beta\mathbf{S}\bullet\mathbf{g}\bullet\mathbf{B} + D\left(\mathbf{S}_{\mathbf{z}}^2 - \frac{S(S+1)}{3}\right) + E\left(\mathbf{S}_{\mathbf{x}}^2 - \mathbf{S}_{\mathbf{y}}^2\right) \\ + \frac{eQV_{zz}}{4}\left[\mathbf{I}_{\mathbf{z}}^2 - \frac{I(I+1)}{3} + \frac{\eta}{3}\left(\mathbf{I}_{\mathbf{x}}^2 - \mathbf{I}_{\mathbf{y}}^2\right)\right] + \mathbf{S}\bullet\mathbf{A}\bullet\mathbf{I} - g_N\beta_N\mathbf{B}\bullet\mathbf{I}\end{aligned} \qquad (1)$$

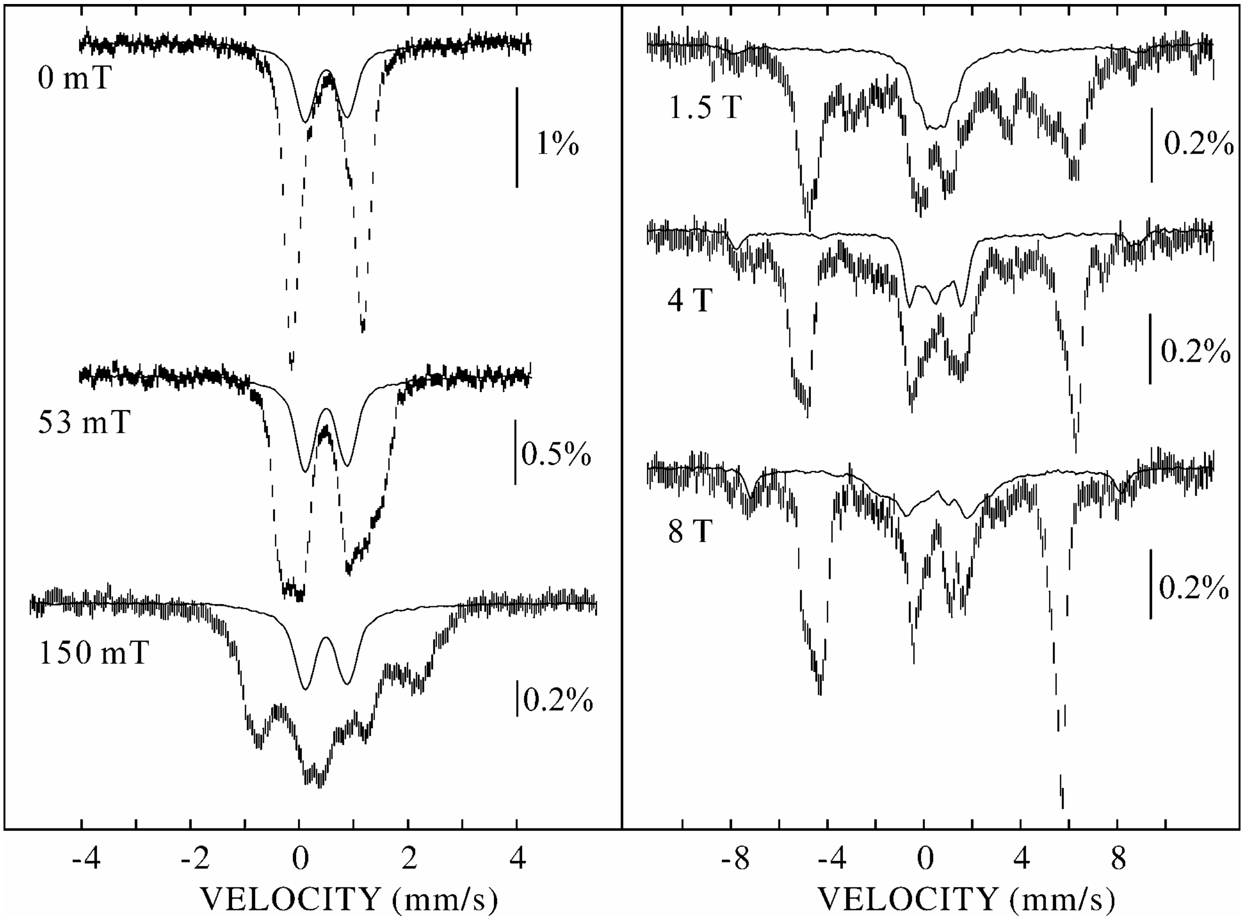

Figure S1: Generation of Mössbauer spectra of the Mn^{IV}/Fe^{III}-R2 complex. The experimental spectra of the sample described in the manuscript are shown as vertical bars. The active Mn^{IV}/Fe^{III}-R2 complex contributes 75% of the total absorption. The remaining 25% of the absorption is contributed by the $Fe_2^{III/III}$-R2 contaminant (solid lines). Removal of this component results in the spectra shown in Figure 2 of the main manuscript.

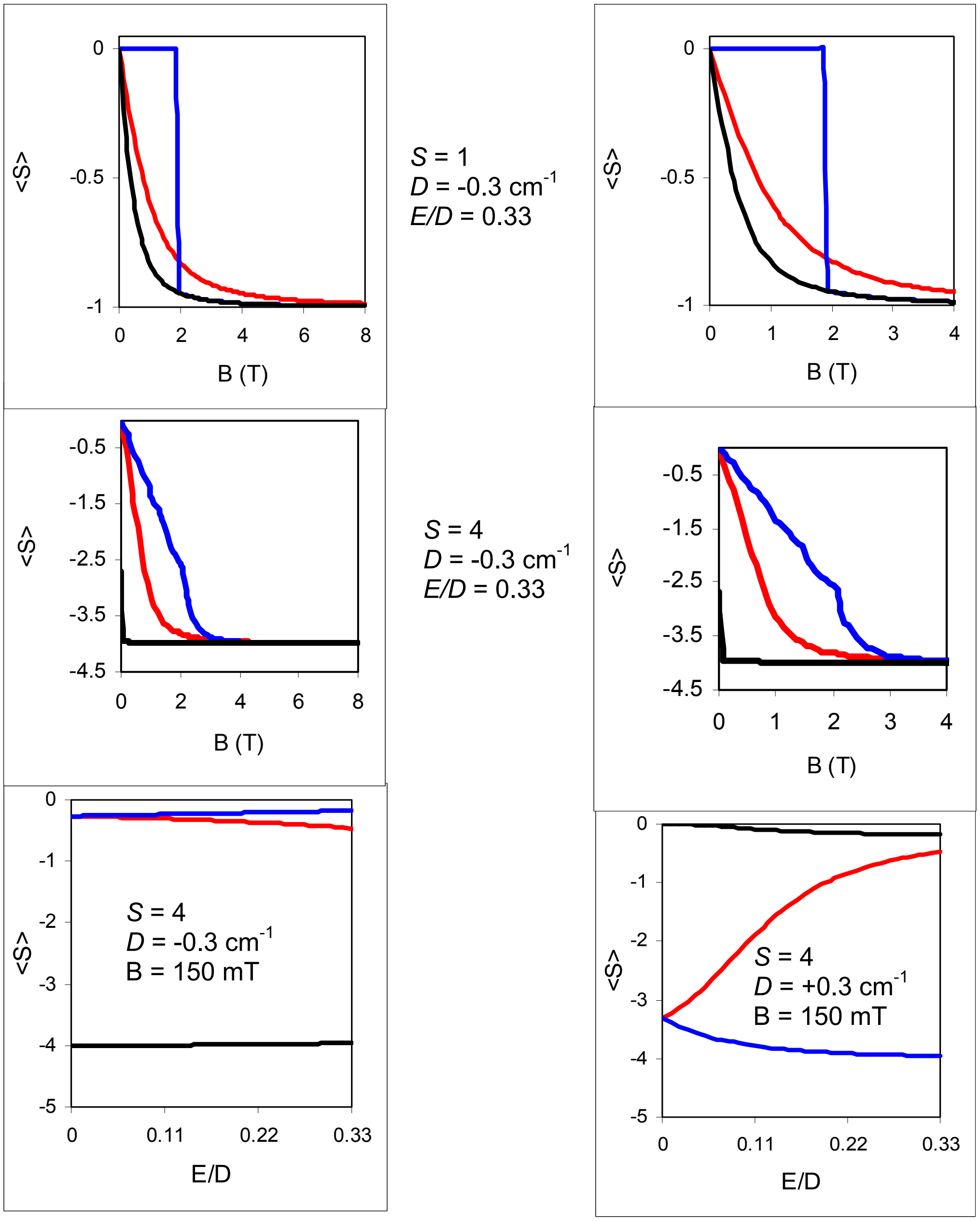

Figure S2: Field-dependence of the spin expectation values of an *S* = 1 spin system (top row, parameters: *D* = -1.9 cm^{-1}, *E*/*D* = 0.33) and an *S* = 4 spin system (middle row, parameters: *D* = -0.3 cm^{-1}, *E*/*D* = 0.33). *E*/*D*-dependence of the spin expectation values of an *S* = 4 spin system at B = 150 mT [bottom row, parameters: *D* = -0.3 cm^{-1} (left) and *D* = +0.3 cm^{-1} (right)]. The spin expectation values of only the ground state are shown. The calculations were carried out with the magnetic field oriented along the x-axis (red), y-axis (blue), or z-axis (black). The *E*/*D* dependence shows that the magnitude of the z-component is large for all *E*/*D* values. For *D* > 0, the component with the large expectation value is in the y-direction. All combinations of ZFS parameters have at least one large value of $\langle S \rangle$.

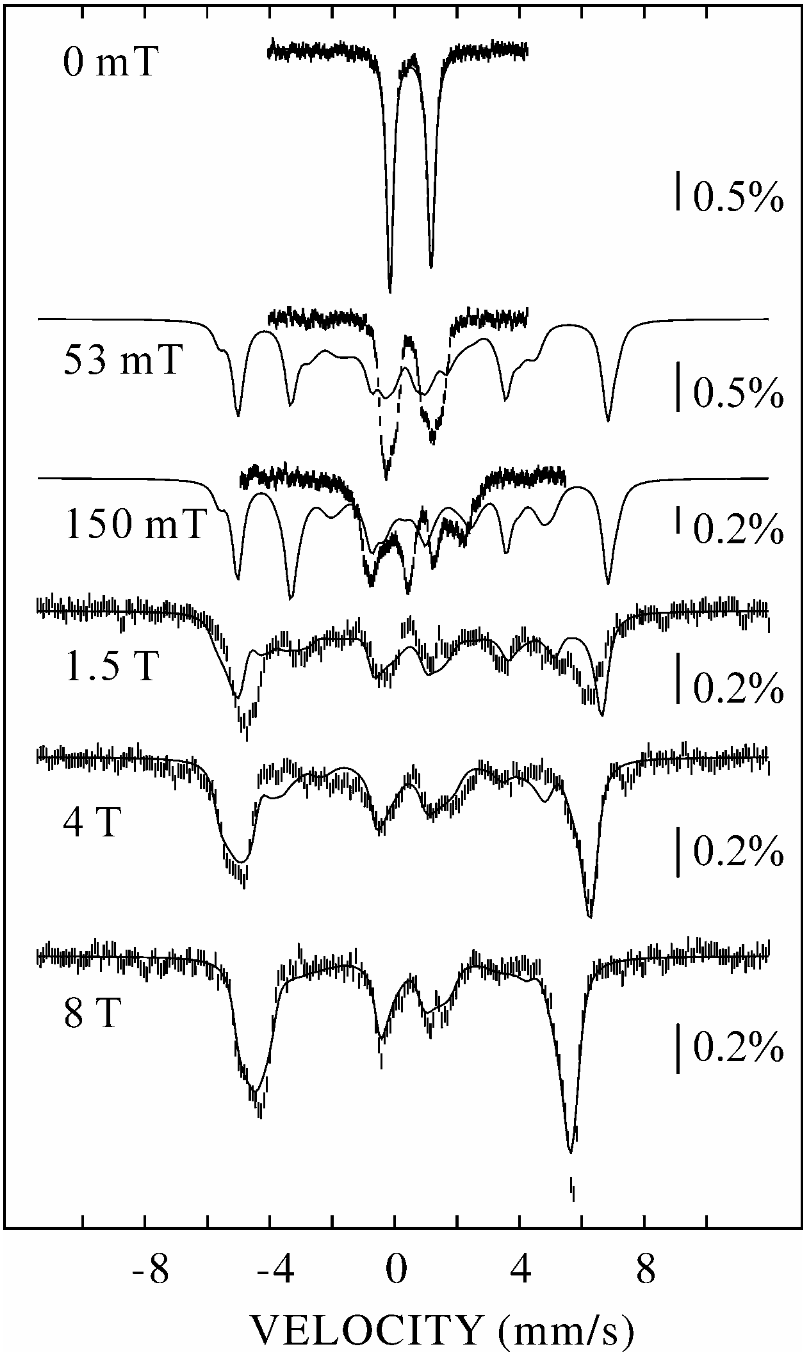

Figure S3: Analysis of the spectra of $\mathrm{Mn^{IV}/Fe^{III}}$-R2 with the assumption of an $S_{\mathrm{Total}} = 4$ ground state and the slow relaxation limit. The simulation parameters were: $S_{\mathrm{Total}} = 4$, $D_{S=4}$ -0.23 cm^{-1}, $(E/D)_{S=4} = 0.33$, $\delta = 0.52$ mm/s, $\Delta E_Q = 1.32$ mm/s, $\eta = -2.2$, $(\mathbf{A}/g_N\beta_N)_{\mathrm{Fe}} =$ (-9.7, -10.0, -9.3) T.

References

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