

Supporting information

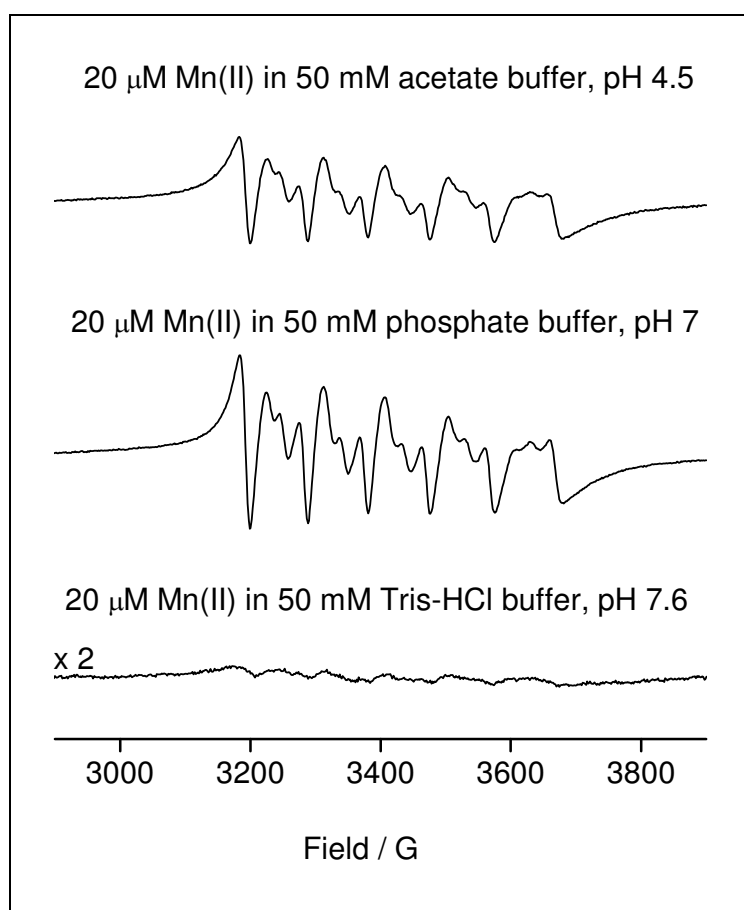
**Metal Binding and Activity of Ribonucleotide Reductase Protein R2 Mutants: Conditions for Formation of the Mixed Manganese-Iron Cofactor**

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The 20 K EPR signal line shape and intensity of Mn(II) ions strongly depends on the buffer (Fig S1). The Mn(II) ions in acetate and phosphate buffers exhibit very similar spectra: they consist of 6 hyperfine splittings centered around  $g = 2$  with the hyperfine coupling constant of 260 MHz which is typical for octahedrally coordinated manganese ions to oxygen ligands. The Mn(II) in Tris-HCl buffer signal also exhibits a 6-line spectrum but much lower in intensity (the signal amplitude is about 20x smaller than in acetate buffer), probably due to less stable coordination of manganese ions in Tris than in acetate or phosphate buffer.



**Figure S1.** X-band (9.5 GHz) EPR spectra at  $(20 \pm 0.4)$  K of 20 μM  $\text{MnCl}_2$  in different buffers: 50 mM acetate buffer, 50 mM phosphate buffer, pH 7.0, and 50 mM Tris-HCl, pH 7.6. Spectra were recorded at non-saturating microwave power level, 3 mW, 0.5 mT modulation amplitude and 100 kHz modulation frequency.