Supplementary material

NrdH-REDOXIN MEDIATES HIGH ENZYME ACTIVITY IN MANGANESE-RECONSTITUTED RIBONUCLEOTIDE REDUCTASE FROM *Bacillus anthracis*

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Figure S1. The *B. anthracis nrdIEF* operon. IVS denotes intervening sequence, and HEG denotes homing endonuclease gene. Primers described in Material & Methods and used for construction of the NrdE expression plasmid pETS146 are shown.

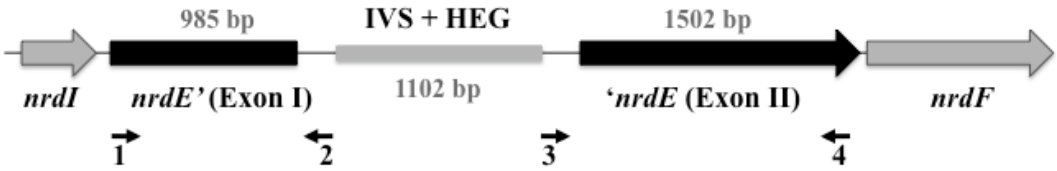

Figure S2. SDS-PAGE of purified *B. anthracis* NrdE. Lane 1, protein ladder (Fermentas); lane 2, crude extract (5 µg) of *E. coli* culture overproducing *B. anthracis* NrdE; lane 3, purified *B. anthracis* NrdE (3 µg).

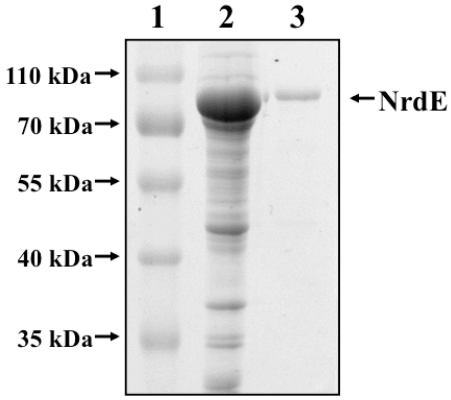

Figure S3. Separation of *B. anthracis* NrdI from Mn-reconstituted NrdF using MonoQ anion exchange chromatography. Separation of the NrdI protein and the NrdF protein with radical and lacking radical is indicated. The absorption is shown in mAU and the gradient from 0-60% 1 M KCl is shown.

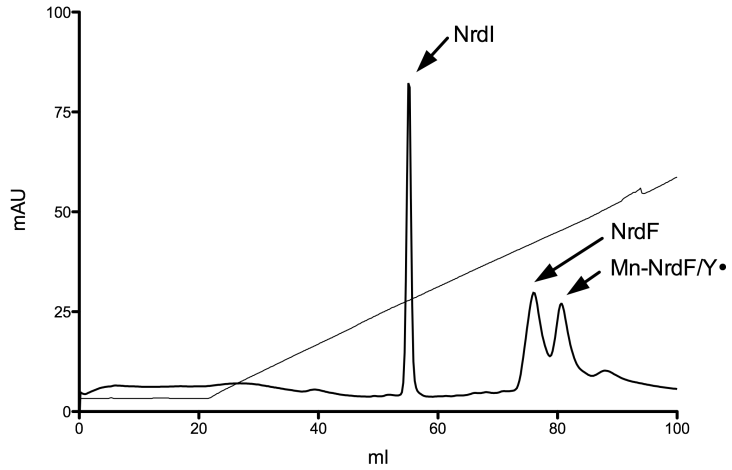

Figure S4. Dithiothreitol and pH dependence of the *B. anthracis* RNR enzyme assay. A) Dependence on dithiothreitol, B) dependence on pH. All incubations were done under standard conditions described in the material and methods using 7.4 µM of NrdE and 16.2 µM of Fe-NrdF.

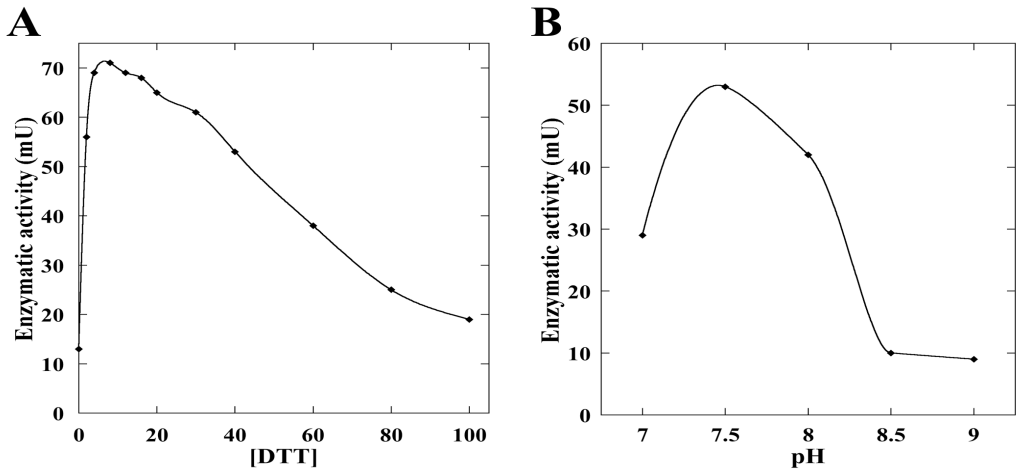

Figure S5. *B. anthracis* NrdI/NrdF interaction SPR sensorgrams and steady state plots. 2.5 μM-60 μM of NrdI was injected over immobilized biotinylated NrdF proteins.

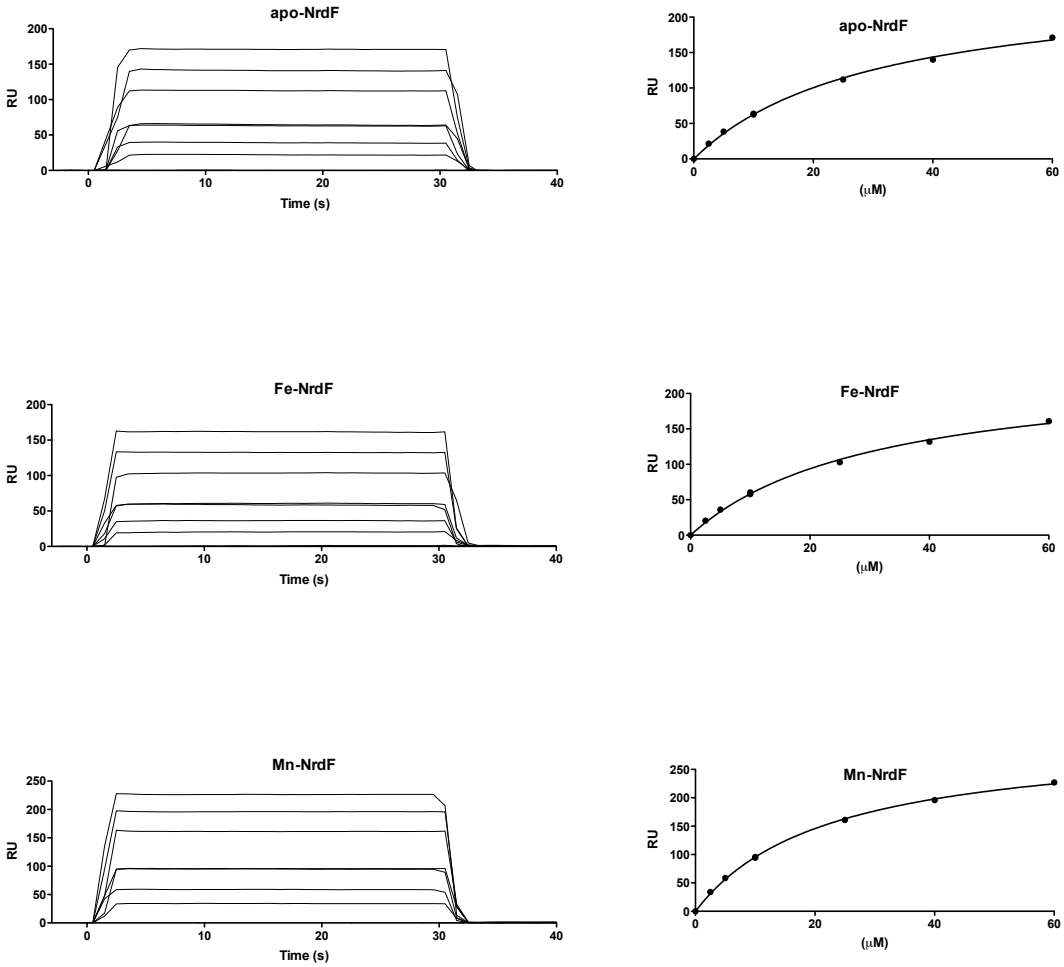

Figure S6. *B. anthracis* NrdE/NrdF interaction SPR sensorgrams and steady state plots. 0.025 μM-2 μM of NrdE was injected over immobilized biotinylated NrdF proteins.

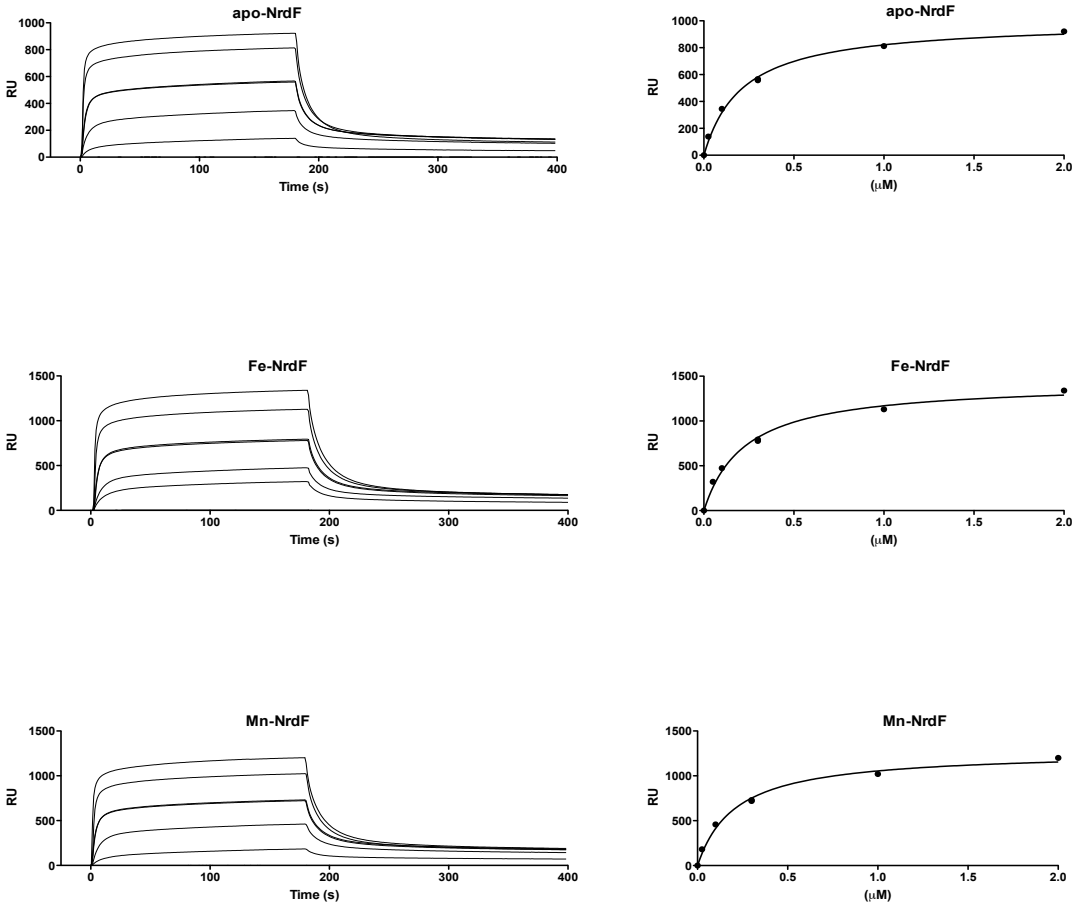

Figure S7. Dimerization of *B. anthracis* NrdE is promoted by effectors but not by substrate. GEMMA analyses of 0.02 mg/ml NrdE alone and in the presence 25 μM dGTP, 50 μM dTTP, or 50 μM CDP. The composition and predicted sizes of the species (in kDa) are indicated.

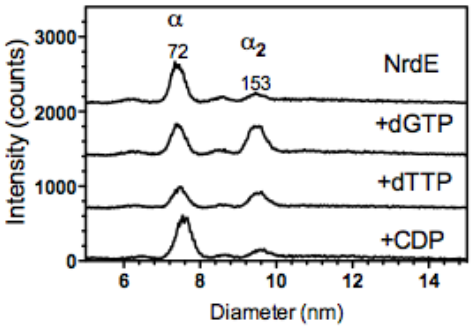

Figure S8. Titration of *B. anthracis* NrdE+dTTP with increasing concentrations of Mn-NrdF. GEMMA analyses of 0.02 mg/ml NrdE, 50 µM dTTP, in the presence of varying concentrations of Mn-NrdF (0.0053 and 0.017 mg/ml). The composition and predicted sizes of the species (in kDa) are indicated.

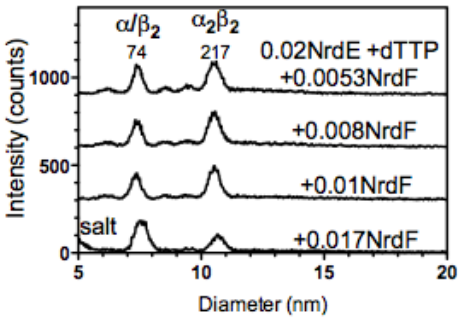

Figure S9. Activity of *B. anthracis* NrdF in presence of increasing NrdI concentrations. The activity of Fe and Mn reconstituted *B. anthracis* NrdF proteins were only marginally effected by an NrdI excess of up to 8 times. Specific activity in absence of NrdI: Mn-NrdF 67.83 U/mg and Fe-NrdF 10.67 U/mg.