Supporting Information for

A Chemically Competent Thiosulfuranyl Radical on the *Escherichia coli* Class III Ribonucleotide Reductase

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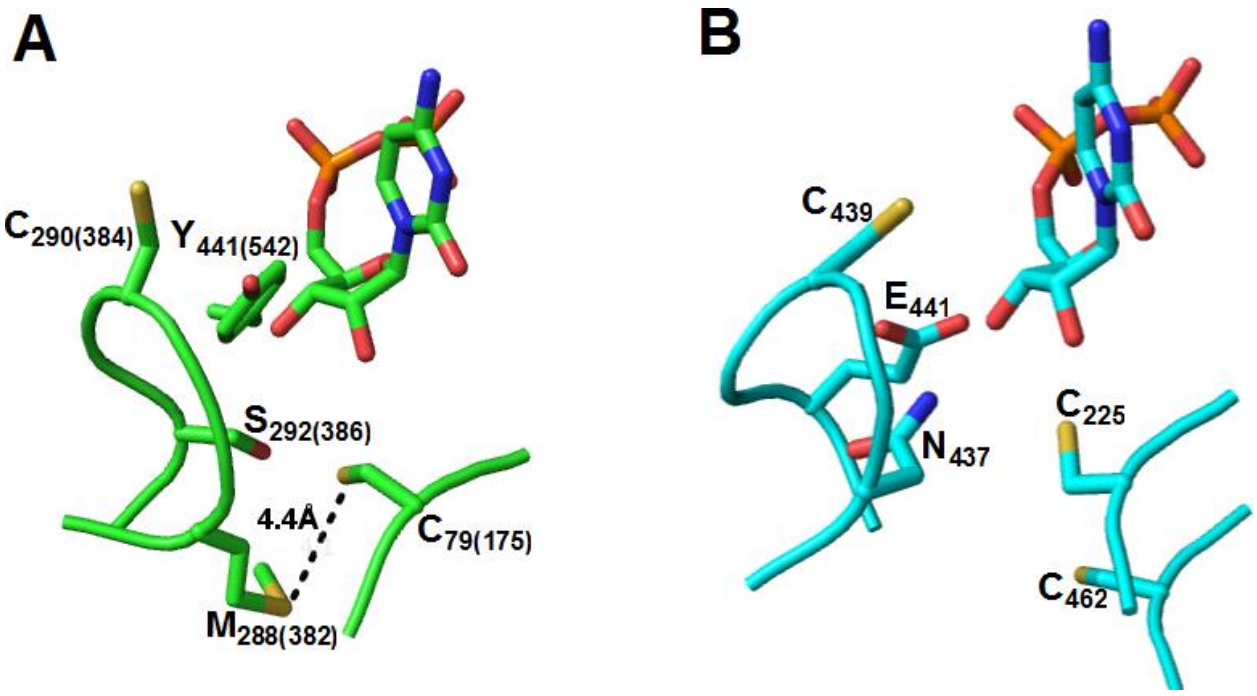


Figure S1. A) Active site from the crystal structure of bacteriophage T4 NrdD G580A mutant (PDB accession ID 1HK8)[1] (*E. coli* NrdD shares a 58% sequence identity with this protein, and its residues are numbered in the parentheses). CDP was docked by aligning the structures of NrdD and CDP-bound *E. coli* NrdA using pymol[2]; B) Active site from the crystal structure of *E. coli* NrdA with substrate CDP. (Zimanyi, Drennan 2013 in preparation)[3].

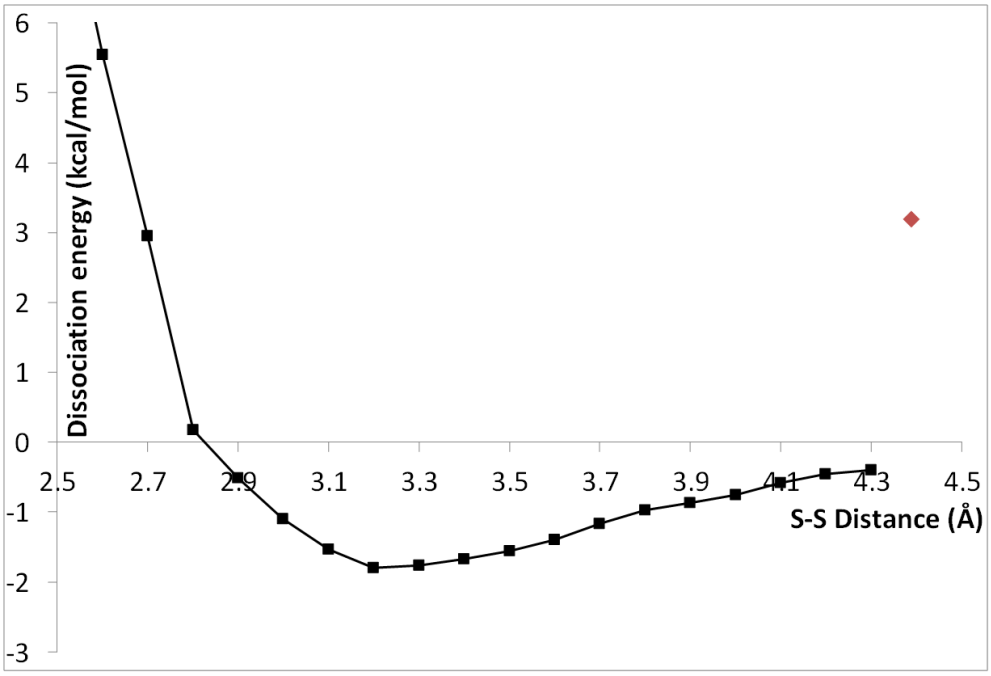
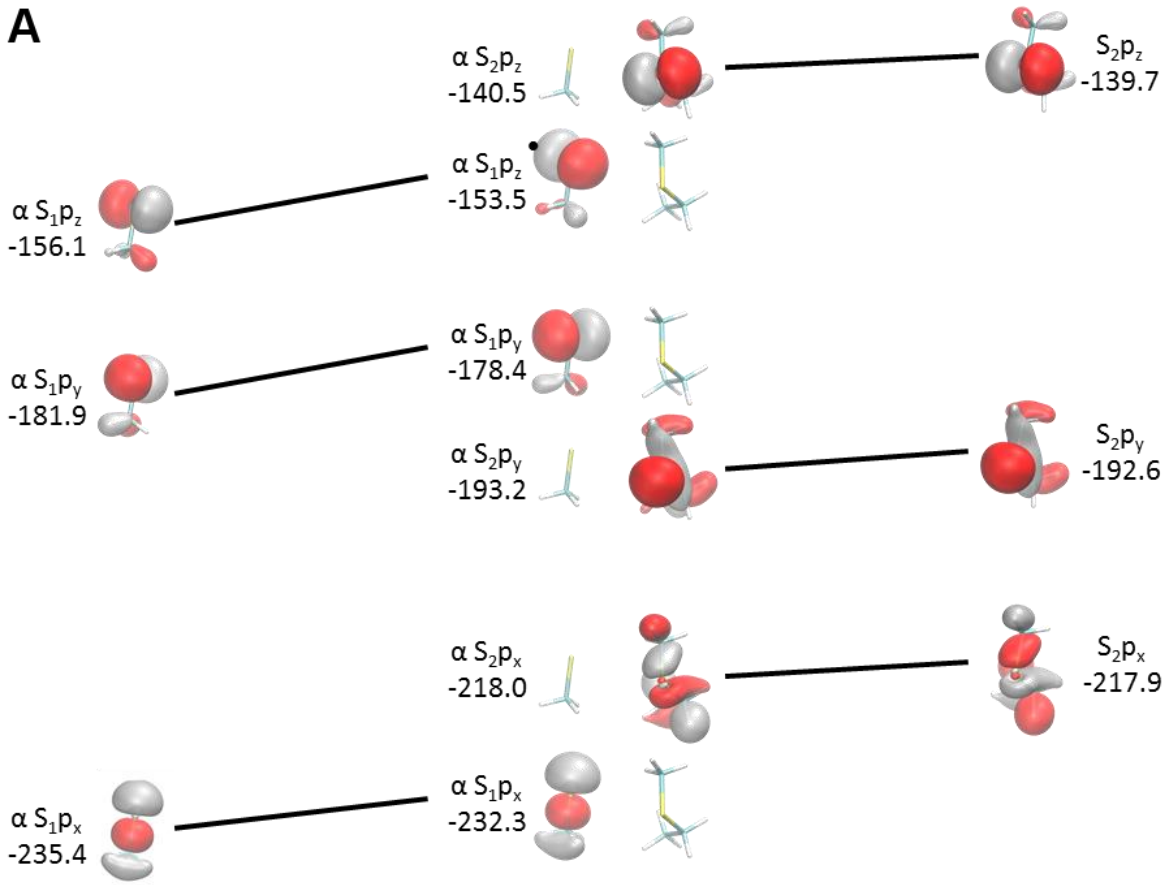


Figure S2. Minimal energy reaction profile along the S_1-S_2 dissociation coordinate (black line with squares) for the model thiosulfuranyl radical formed from methanethiyl radical and ethylmethylsulfide. Shown for comparison is the native structure (red diamond), which does not lie on the minimal energy reaction coordinate.



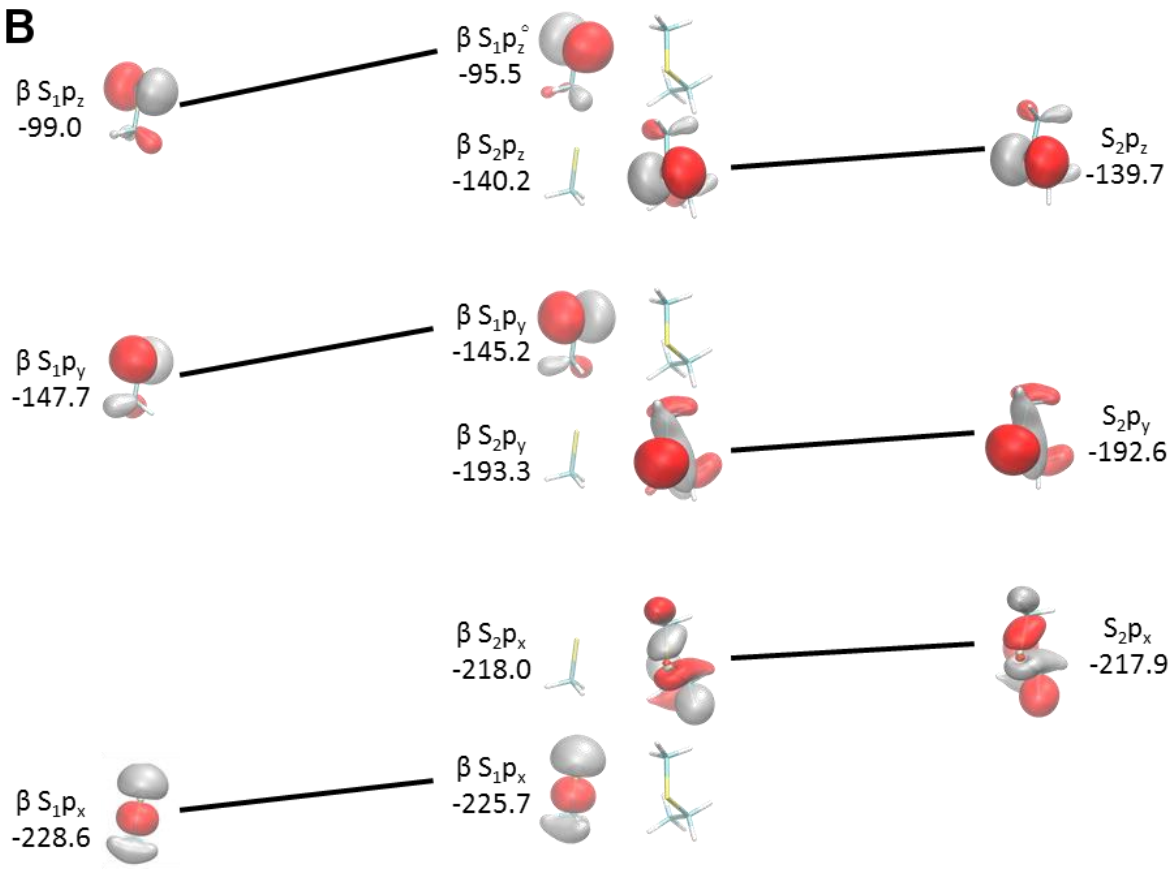
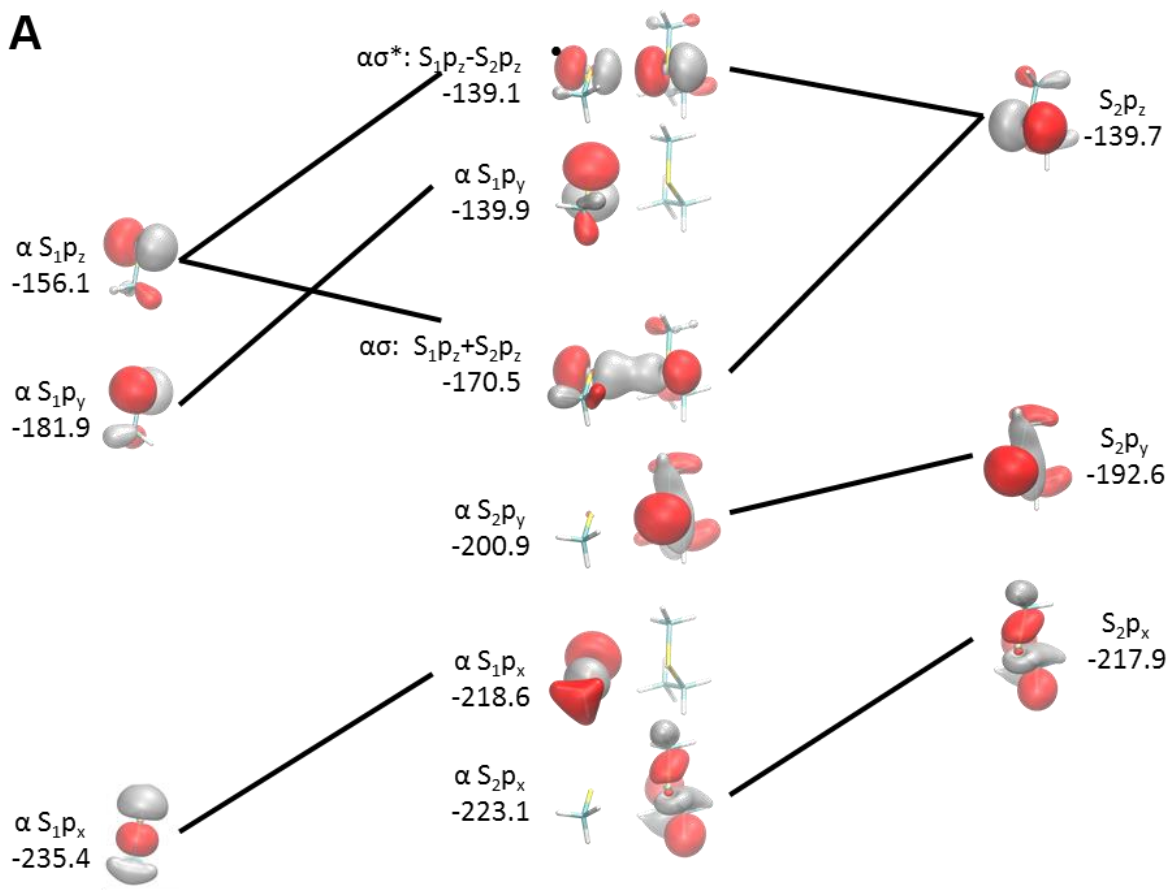


Figure S3. Frontier molecular orbitals for methanethiyl radical and ethylmethylsulfide constructed using coordinates from the crystal structure of T4 bacteriophage NrdD, showing essentially no interaction between the methanethiyl and ethylmethylsulfide moieties. Diagrams for the α (A) and β (B) spin orbitals are shown separately, together with orbital energies in kcal/mol, which represent the ionization enthalpy of the electron in that orbital. For each diagram, the leftmost and rightmost orbitals represent the fragment molecular spin orbital of the methanethiyl and ethylmethylsulfide fragments respectively. S_np_z refers to the $3p_z$ orbital on the sulfur atom of fragment n (1=methanethiyl, 2=ethylmethylsulfide).


A
ασ*: S1pz-S2pz
-139.1
α S1py
-139.9
ασ: S1pz+S2pz
-170.5
α S1pz
-156.1
α S1py
-181.9
α S2py
-200.9
α S1px
-218.6
α S2px
-223.1
α S1px
-235.4
S2pz
-139.7
S2py
-192.6
S2px
-217.9

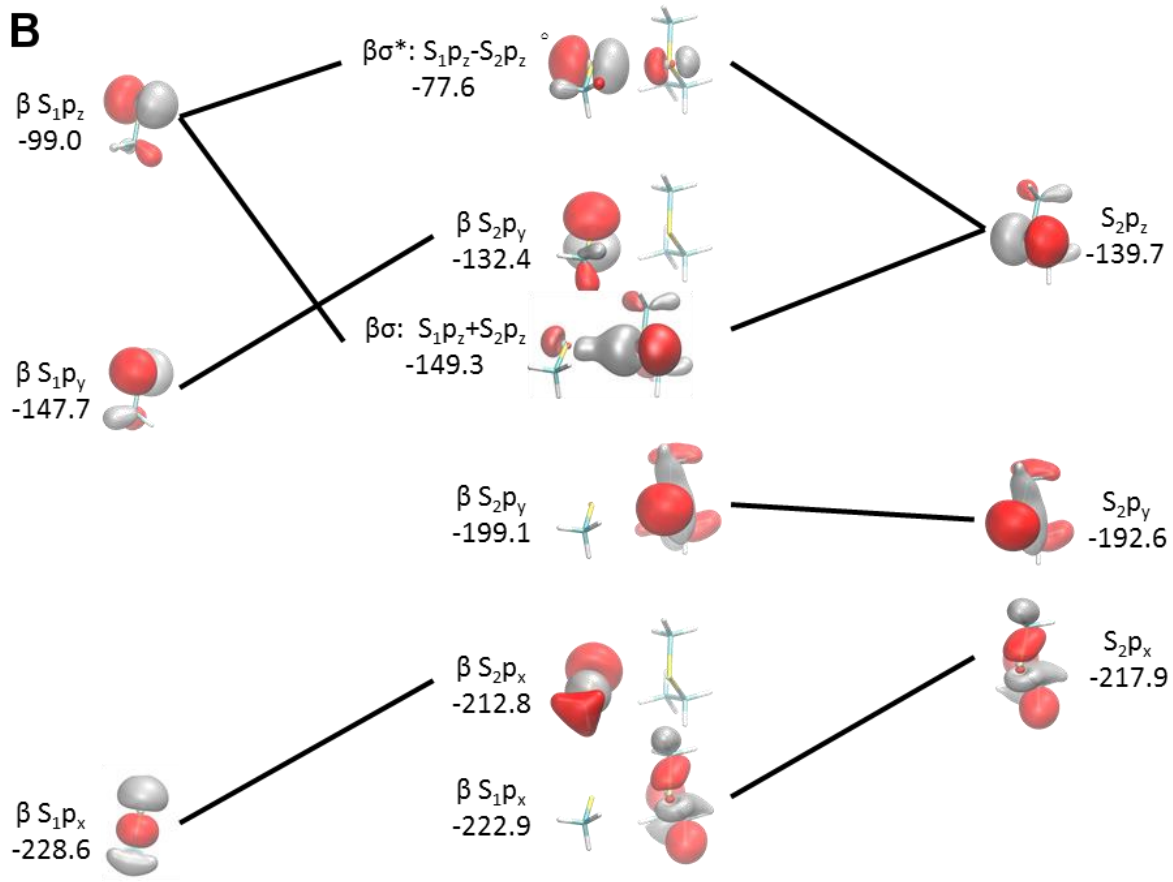


Figure S4. Frontier molecular orbitals for the model thiosulfuranyl radical formed from methanethiyl radical and ethylmethylsulfide. Diagrams for the α (A) and β (B) spin orbitals are shown separately, together with orbital energies in kcal/mol, which represent the ionization enthalpy of the electron in that orbital. For each diagram, the leftmost and rightmost orbitals represent the fragment molecular spin orbital of the methanethiyl and ethylmethylsulfide fragments respectively, which mix to produce the σ bonding and σ^* antibonding orbitals. S_np_z refers to the $3p_z$ orbital on the sulfur atom of fragment n (1=methanethiyl, 2=ethylmethylsulfide).

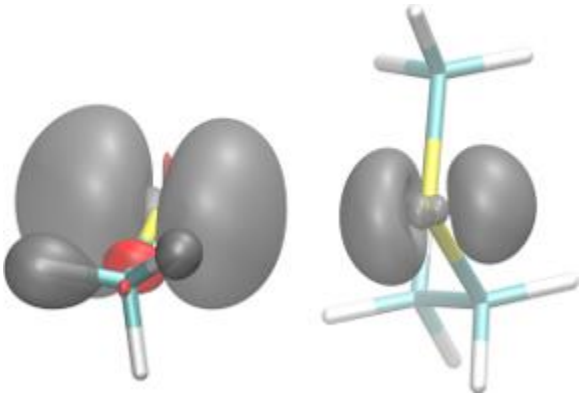


Figure S5. Excess spin densities for the minimal energy model, showing significant excess α spin on the ethylmethylsulfide sulfur. Key: grey lobes, α spin excess; red lobes, β spin excess.

	Condition	Product	Trial 1	2	3	4	5	Mean	Stdev
A	(-)HCO_2^-	Cyt	1.16	1.04	0.94	1.05	0.79	1.00	0.14
B	(-)HCO_2^-	dC	0.03	0.24	0.43	0.55	0.64	0.38	0.25
C	(+)HCO_2^-	Cyt	0.14	0.16	0.09	0.10	0.09	0.12	0.03
D	(+)HCO_2^-	dC	1.31	1.70	1.51	1.49	1.84	1.57	0.21
E	Cyt consumed		1.02	0.89	0.85	0.95	0.70	0.88	0.12

Figure S6. Products formed after quenching of the thiosulfuranyl radical generated by reaction of NrdD with 5-[^{3}H]-CTP and ATP, as described in Figure 8A and in text. Results for 5 separate trials are given here to show variability between replicates, averages are given Table 2. Amount of 5-[^{3}H]-Cyt (A,C) and 5-[^{3}H]-dC (B,D) formed per G• after quenching the thiosulfuranyl radical in the absence (A,B) or presence (C,D) for formate. Addition of formate results in the consumption of 5-[^{3}H]-Cyt (E = A−C), being converted into 5-[^{3}H]-dC.

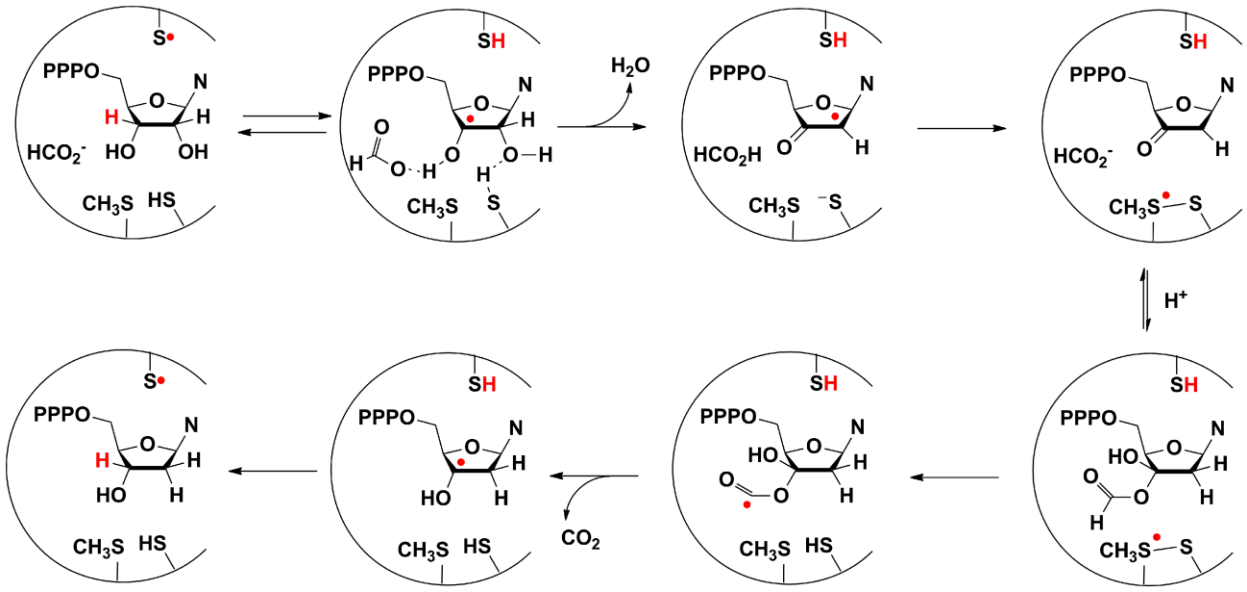


Figure S7. Alternative mechanism for nucleotide reduction by NrdD.

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T4                  VG----------------RDIGREILTKMNAHLKQWTERTGFAFSLYSTPAENLCYRFCK 454
Ecoli               FGGEHVYDNE------QLRAKGIAIVERLRQAVDQWKEETGYGFSLYSTPSENLCDRFCR 555
Llactis             YG-PTWENNP------EAKAFTIEIVKRMHEDCEDWSKASGYHYSVYSTPSESLTDRFCR 567
Mjannaschii         LG-EELHESK------DAVKFGEKVIEYIREYADKLKEETGLRWTVTQTPAESTAGRFAR 612
Paeruginosa         SDDREGLHSE------AGREMALALLDHVRARLVGFQEDSGHLYNLEATPAEGTTYRFAR 547
Pfuriosus           LNSPELWKEGNRRDWIEAARLMKRMVEFATEKAREWMKATRVPWNVEEVPGESAQAKLAL 460
Tmaritima           GLTTEDIDGLKYTE--EGEVFVDNVLDTIREEAEKGYHEYGFTFNIEQVPAEKAAVTLAQ 508
Kstuttgartensis     TG-KELHEGD------DMIRQGLRVVSHMYTRVKEAGKKHKLKFSLEESPAESASRRLAK 641
Tacidaminovorans    TG-HELHEDK------GALDLALSVIKYMELKCDQLSERYGVKMVLEQTPAESTAHRFAK 556
Aveneficus          MG-EELHEP-------SAWRFGLEIIKHMMDIATEWSKETGLRWVITQTPAESTAHRFAK 550
Mbarkeri            TG-YQIHESP------VAYKLAIRAMFEMKMHAQKLSKETGMEIALARTPAETTAQRFAV 626
                                          :           .       :   *.*     :.

T4                  -YDIKQLALECASKRMYPDIISAKNNKAITGSSVPVSPWGCRSFLSVWKDSTGNEILD-- 307
Ecoli               -YDIKQLALECASKRMYPDILNYDQVVKVTGS--FKTPWGCRSFLGVWENENGEQIHD-- 401
Llactis             -YDIKELALECSTKRMYPDILSYDKIVELTGS--FKASWGCRSFLQGWKDENGNDVTA-- 415
Mjannaschii         NKELMYKIHQLSAKFGIPYFINMLPDWQVT----NTNAWGCRTRLS--GNWTGDAEID-- 465
Paeruginosa         --DNATRLFEMTARYGLPYFQNFLNSDMQPNQ---VRSMCCRLQLDVRELLKRGNGLFG- 404
Pfuriosus           ---VFEAIFTTAAKRGSFYWLNTNVVDPDASY-----AMCCRLNIDKREFTYTFSLDEDV 296
Tmaritima           -----------SARFINKINMKWQDTNWYISDSIDAVASCCRLTSSTQTLKKFSLSSEEE 349
Kstuttgartensis     QYELLEYACQIASENGVPYFVFDR------DE-IT-LSACCRLRTTIDDN--YMIKHP-- 490
Tacidaminovorans    WEEFLELACRVASEKGNTYFVFDR------GG-VAKLSECCRLSFELSEEDLREAHQP—406
Aveneficus          FDEFMILVHKCVAKYGTPYFLNLLAGYLPD----NVFAQCCRLVLSPDANDWEDFAKG-- 406
Mbarkeri            YKELYRMTFELAAKFGTPYFDNQLPEYRGAGE-GISCYQCCAYQFS--ANPTDDKEFDDK 466
                                                      *
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Figure S8. NrdD alignments created using ClustalW[4]. Blue text denotes formate-utilizing NrdDs. Black and red text denote putative disulfide-utilizing NrdDs. Red text denotes NrdDs with a thioredoxin in the operon. Putative residues involved in chemistry highlighted in green.

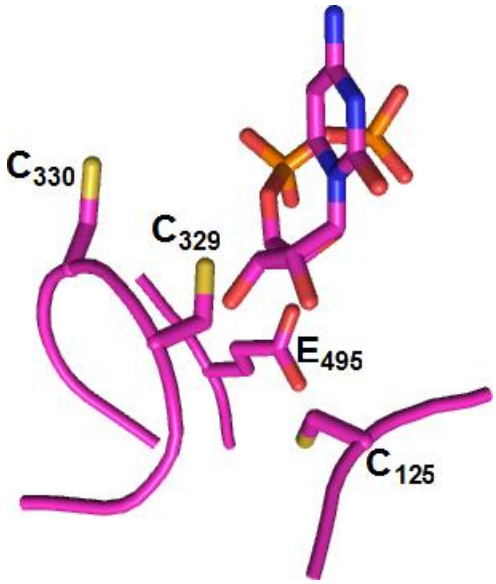

Figure S9. Homology model of *Thermotoga maritima* NrdD created using Phyre2.[5] Residues C330 and C125 are conserved in all RNRs. In addition, residues C329 and E495 are conserved in NrdDs related to *T. maritima* NrdD. We hypothesize that nucleotide reduction by *T. maritima* NrdD is accompanied by formation of a disulfide bond between C125 and C329.

References

(1) Logan, D. T.; Andersson, J.; Sjöberg, B.-M.; Nordlund, P. *Science* **1999**, *283*, 1499.

(2) Schrödinger, LLC 2010.

(3) Zimanyi, C. M. [PhD Thesis]; Massachusetts Institute of Technology: Cambridge, 2013.

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(5) Kelley, L. A.; Sternberg, M. J. *Nat. Protoc.* **2009**, *4*, 363.