Supporting Information for:

A Substrate Radical Intermediate in Catalysis by the Antibiotic Resistance Protein Cfr

Tyler L. Grove[1], *Jovan Livada*[1], *Erica L. Schwalm*[1], *Michael T. Green*[1], *Squire J. Booker*[1,2] *and Alexey Silakov*[1]

The Departments of [1]Chemistry and of [2]Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802.

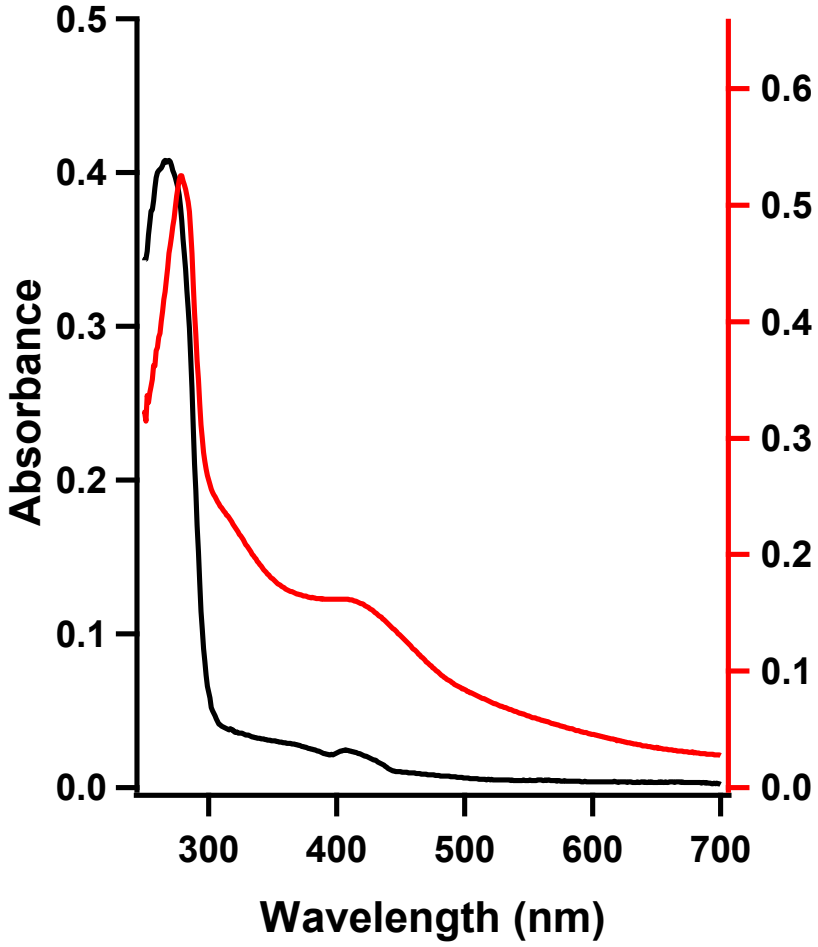
Supplementary Results.

Supplementary Table 1. Comparison of HF coupling constants (in MHz) obtained experimentally or calculated for two models that differ by the protonation state of the N7 atom.

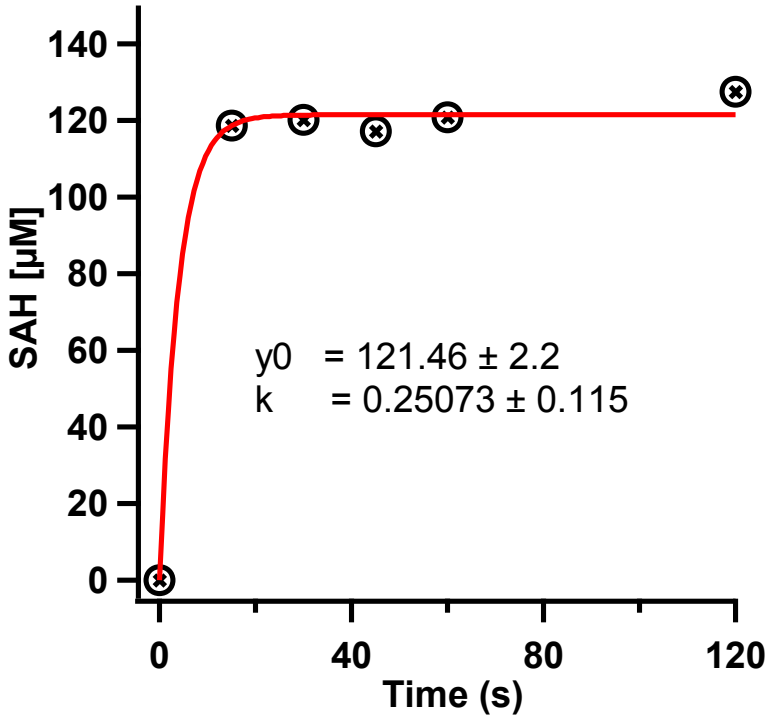
nucleus	Experiment				DFT, non-protonated				DFT, protonated			
	A_x*	A_y*	A_z*	A_{iso}	A_x	A_y	A_z	A_{iso}	A_x	A_y	A_z	A_{iso}
C8-H	80	82±2	85	82.3	+86.4	+88.0	+95.6	**+90.0**	+80.6	+82.3	+89.2	**+84.0**
C2-H	15	14	13±1	12.7	-23.4	-15.7	-6.1	**-15.1**	-13.7	-9.5	-2.6	**-8.6**
NH_2	9	9	7±2	8.3	-15.3	-10.4	+0.9	**-8.3**	-20.5	-14.1	0.1	**-11.5**
NH_2	6.5	6.5	4±2	5.7	-11.5	-11.2	-2.1	**-8.2**	-16.6	-14.3	-1.8	**-10.9**
1'-H	2	2	-2:0**	0.1:0.7**	-1.5	-1.3	+2.8	**-0.0**	-1.2	-0.8	+3.8	**+0.6**
C8-^{13}C	60	60	64	61.3	+59.6	+59.9	+70.6	**+63.4**	+42.7	+43.0	+57.0	**+47.5**
N7	60±3	-5±2	-5±2	16.7	+61.5	-4.5	-5.0	**+17.3**	+45.9	-2.7	-2.8	**+13.5**
C8-CH_2					-6.1	-4.4	1.9	**-2.9**	-4.8	-2.8	+2.4	**-1.7**
C8-CH_2					-6.0	-4.5	1.6	**-3.0**	-5.6	-3.9	1.2	**-2.8**
N^7-H					-	-	-	**-**	-45.0	-31.4	-2.0	**-26.1**

*If not specified, the uncertainty in the determination is ±0.5 or better in the unit of the last significant digit. If uncertainty is larger, a number is provided

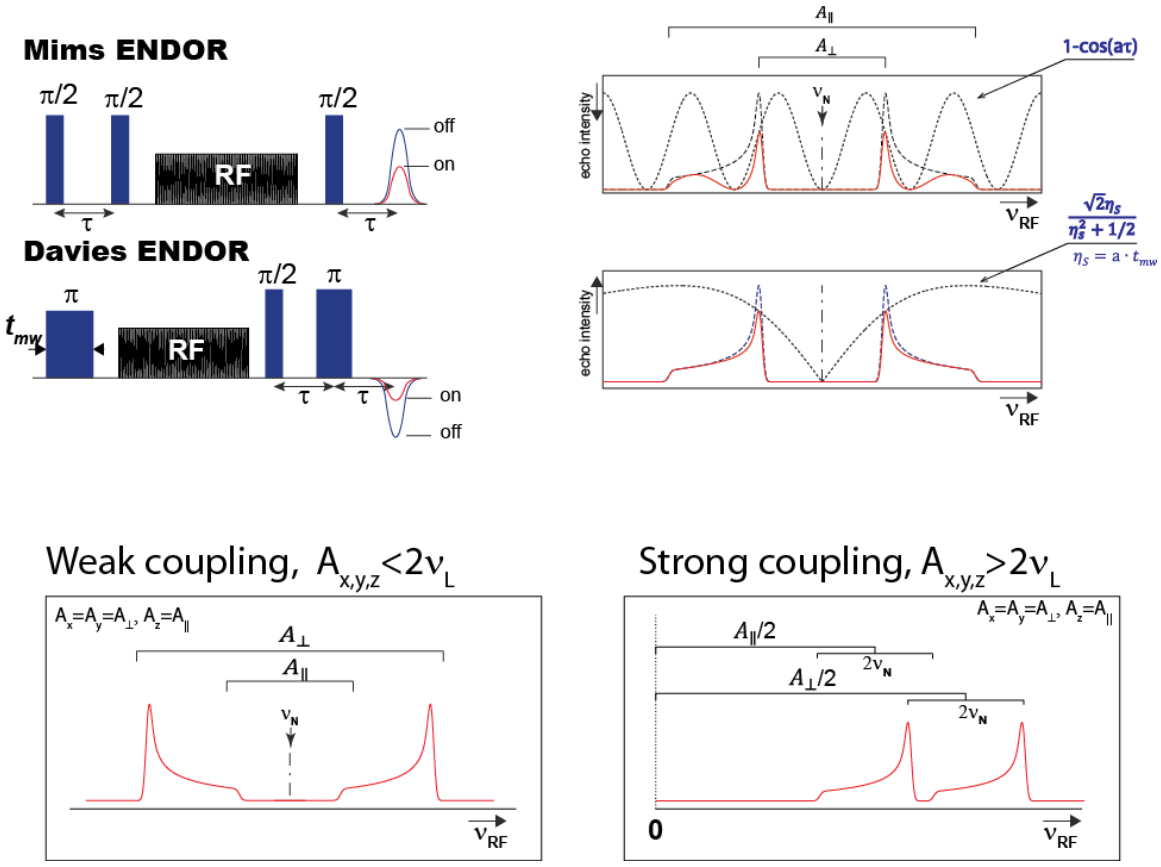
**A_3 part of the coupling could not be identified in our experiment



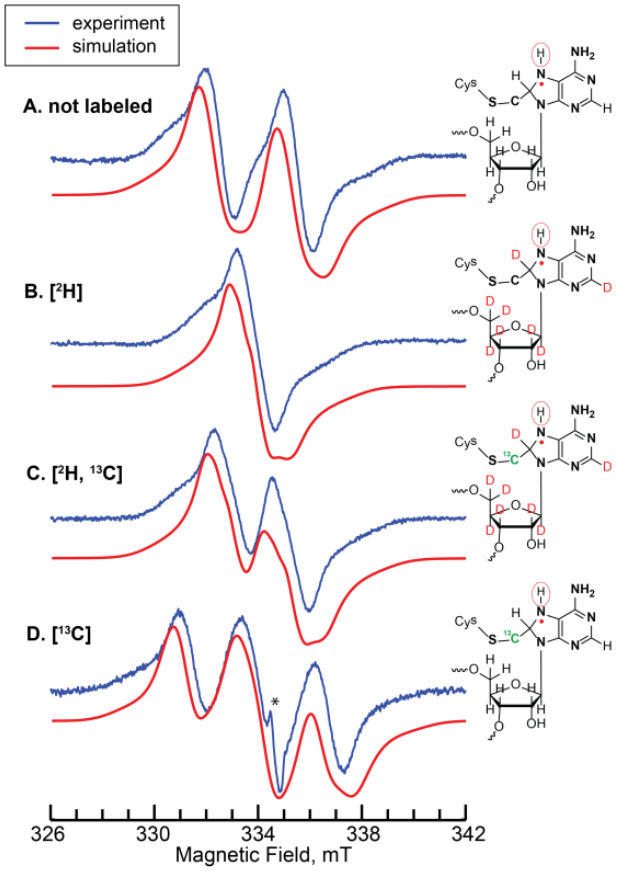
Supplementary Figure 1. A) UV–vis traces of wt Cfr_{apo} (5.1 μM, solid black line) and wt $Cfr_{apo\text{-}RCN}$ (7.1 μM, solid red line).



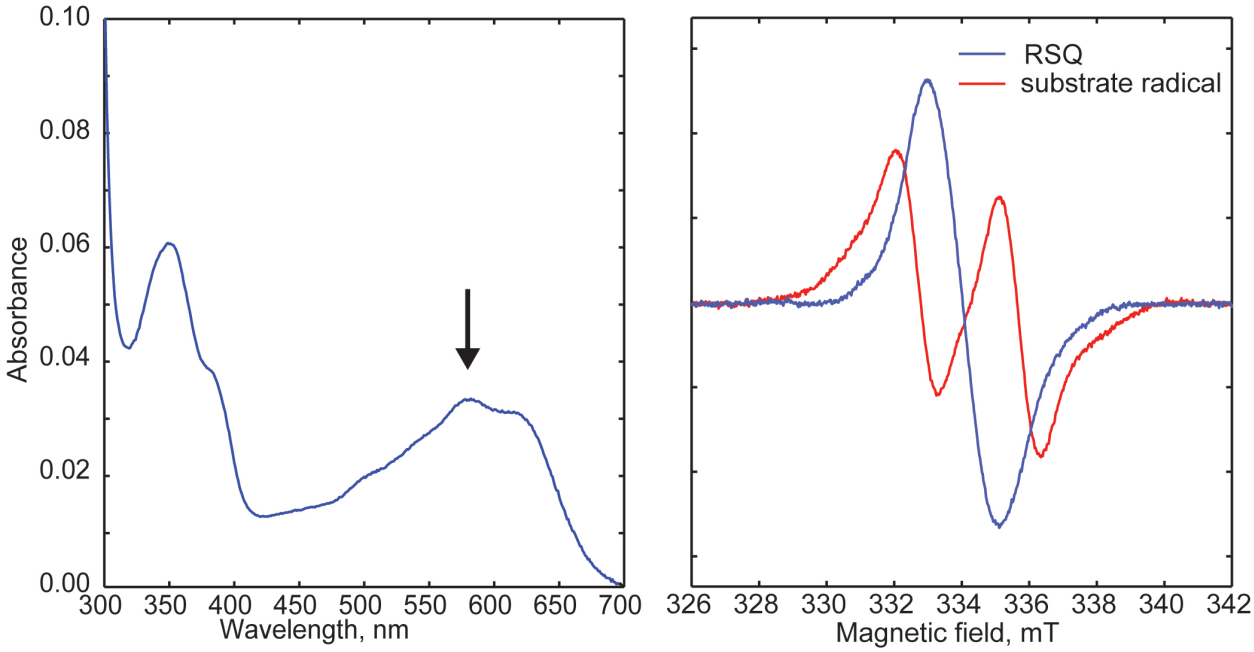
Supplementary Figure 2. Methyl transfer catalyzed by wt $Cfr_{apo\text{-}RCN}$ (150 µM) in the presence of 2 mM SAM. The data were fitted to a pseudo-first order exponential equation (red line).



Supplementary Figure 3. Mims and Davies pulse sequences used in this work (upper left), and illustration of distortions induced by the respective sequences relative to a hypothetical axial ENDOR spectrum (S=1/2, I=1/2, $A_z=A_{II}> A_x=A_y=A_\perp$) . The lower part of the figure illustrates peak positions for two cases: weak ($2\nu_L> [A_\perp > A_{II}]$) and strong ($2\nu_L< [A_\perp > A_{II}]$) hyperfine coupling. This distinction arises from the fact that in standard ENDOR experiments no information about the sign of the corresponding nuclear transition frequency is obtained. Thus, in the strong coupling case, negative frequency signals are "folded" to the positive axis.



Supplementary Figure 4. Simulation of the CW EPR spectra, presented in Figure 1, accounting for a strongly coupled proton at N7 (see Supplementary Table 1 for the corresponding HF coupling constants), according to the protonated model.



Supplementary Figure 5. UV-visible spectrum of flavodoxin semiquinone (left) and the corresponding CW-EPR spectrum (labeled RSQ) recorded at 100 K (right, blue trace). The arrow above 580 nm indicates the peak at which the concentration of the semiquinone standard was determined. CW-EPR spectra were acquired as described in the Materials and Methods.

Supplementary Note 1

Coordinates:

Non-protonated model (presented in the main text):

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BP86 optimization, final energy -1441.36456 A.U.

C	-4.8477670	-1.3796160	0.6812370
C	-3.6775200	-1.3609030	-0.1443480
C	-3.2580830	-0.0675260	-0.5985030
C	-5.0572880	0.8484960	0.5286960
H	-5.6078810	1.7566200	0.7902710
N	-2.1694800	0.0803810	-1.3829770
H	-1.8983030	1.0046260	-1.7051940
H	-1.6232710	-0.7279460	-1.6633970
N	-3.9738090	1.0266690	-0.2416960
N	-5.5613540	-0.3078680	1.0295600
N	-3.1652580	-2.5688330	-0.3875660
N	-5.0690890	-2.6925520	1.0264110
C	-4.0073520	-3.5086370	0.3503560
H	-4.4927660	-4.2164820	-0.3483000
C	-3.0925300	-4.2996610	1.3162130
H	-2.3014420	-4.7469060	0.6990770
H	-2.6212350	-3.6118380	2.0325950
C	-4.1562780	-4.9279220	3.8969580
H	-4.9592710	-4.1811760	3.8703410
H	-4.4591470	-5.7506500	4.5580730
S	-3.8658840	-5.6907950	2.2493660
C	-8.5623600	-2.9412420	0.6693060
O	-7.2312760	-3.2173290	0.2031450
C	-6.3519710	-3.2579770	1.3748390
C	-7.1191130	-2.5705990	2.5394540
C	-8.3508590	-1.9684490	1.8394770
H	-9.0443010	-3.8640800	1.0499360
H	-6.1702500	-4.3091320	1.6487660
H	-8.0898550	-0.9707060	1.4486690
O	-9.4509420	-1.9006750	2.7538650
H	-10.2544820	-1.8598790	2.1915570
C	-9.4023010	-2.3648390	-0.4592710
H	-9.4850780	-3.0802470	-1.2956500
H	-8.9410560	-1.4322370	-0.8269250
O	-10.7006180	-2.1059160	0.1289690
H	-11.1772090	-1.4845030	-0.4489260
O	-7.5196560	-3.5847330	3.4722130
H	-8.4217790	-3.3216100	3.7597210

H	-6.5036620	-1.8052170	3.0360980
H	-3.2269960	-4.4848960	4.2779200

Protonated model:

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BP86 optimization, final energy -1441.79258 A.U.

C	-4.8263630	-1.3672660	0.5729120
C	-3.6224870	-1.3104660	-0.1970420
C	-3.1818930	-0.0173780	-0.6171870
C	-5.0615580	0.8533020	0.4220770
H	-5.6421480	1.7508820	0.6540160
N	-2.0624800	0.1955220	-1.3297390
H	-1.8293480	1.1519000	-1.5909860
H	-1.4282740	-0.5452180	-1.6108140
N	-3.9447820	1.0516460	-0.2838640
N	-5.5688680	-0.3129890	0.8889410
N	-3.1779540	-2.5715370	-0.3819900
N	-5.0646520	-2.6784400	0.8960910
C	-4.0057420	-3.5394630	0.3431450
H	-4.4361860	-4.2782940	-0.3541940
C	-3.1153310	-4.2756780	1.3980890
H	-2.2548560	-4.6786520	0.8456740
H	-2.7506310	-3.5535280	2.1411900
C	-4.2418560	-5.0293080	3.9175930
H	-5.0342060	-4.2706120	3.9028120
H	-4.5897210	-5.8898790	4.5041360
S	-3.8988250	-5.7009890	2.2423810
C	-8.5707470	-2.9464800	0.6858810
O	-7.2437230	-3.1978570	0.1740710
C	-6.3485460	-3.2339190	1.3096990
C	-7.0579360	-2.5175030	2.4952630
C	-8.3378640	-1.9581270	1.8404090
H	-9.0101750	-3.8793610	1.0881890
H	-6.1508760	-4.2785120	1.6009040
H	-8.1285840	-0.9553330	1.4312930
O	-9.3890350	-1.9200350	2.8048780
H	-10.2236350	-1.8723240	2.2910890
C	-9.4601080	-2.3988730	-0.4189290
H	-9.5814060	-3.1361640	-1.2307480
H	-9.0153760	-1.4765080	-0.8321640
O	-10.7216000	-2.1263840	0.2298910
H	-11.2832470	-1.6229880	-0.3856470
O	-7.3797620	-3.5085550	3.4744570
H	-8.2772690	-3.2715060	3.7987380
H	-6.4317690	-1.7248760	2.9323980
H	-3.3204230	-4.6356370	4.3638430

H	-2.3371160	-2.8581630	-0.8800060