SUPPLEMENTAL INFORMATION

Characterization of a Cross-linked Protein–Nucleic Acid Substrate Radical in the Reaction Catalyzed by RlmN

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Table S1: Primers for constructing the RlmN and Cfr variants*

Primer		Sequence
RlmN C118S	Forward	5'- cga ccg tgc cac gct c**tc c**gt ctc ttc gca ggt ggg g -3'
	Reverse	5'- ccc cac ctg cga aga gac **gga** gag cgt ggc acg gtc g -3'
Cfr C105A	Forward	5'- gga aag ctt t**gc t**at tag cag cca gtg tgg -3'
	Reverse	5'- cca cac tgg ctg cta at**a gc**a aag ctt tcc -3'
RlmN E105A	Forward	5'- ggc gat cag cgc gtc **gca** acg gtg tat atc ccg g - 3'
	Reverse	5'- ccg gga tat aca ccg t**tg c**ga cgc gct gat cgc c – 3'

*Bold and underlined nucleotides indicate the variant codon.

Table S2: Fe and S^{2-} content and UV-vis characteristics of apo RlmN C118S						
	A_{280}/A_{400} ratio		Fe per polypeptide		S^{2-} per polypeptide	
	AI	RCN	AI	RCN	AI	RCN
$RlmN_{C118S}$	NA	2.75	0.184	2.732	0.231	3.745

Table S3. Data collection and refinement statistics for C118A RlmN and C118A RlmN+SAM X-ray structures.

	C118A RlmN+SAM	C118A RlmN
Data collection		
Wavelength	0.97857 Å	0.97857 Å
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	55.627, 55.729, 254.032	55.438, 55.384, 262.377
Resolution (Å)	30.00-2.58 (2.65-2.58)	50.00-2.20 (2.24-2.20)
R_{sym} or R_{merge}	0.116 (0.536)	0.075 (0.503)
$I / \sigma I$	16.6 (2.2)	19.5 (2.5)
Completeness (%)	95.7 (85.5)	96.3 (96.7)
Redundancy	5.9 (3.7)	5.7 (4.7)
Refinement		
Resolution (Å)	30.00-2.58	50.00-2.20
No. reflections	23121	38412
R_{work} / R_{free}	0.263/0.299	0.2055/0.2435
No. atoms		
Protein	5453	5552
Ligand	70	16
Water	0	231
B-factors		
Protein	58.0	34.4
Ligand	58.0	33.9
Water	N/A	40.0
r.m.s. deviations		
Bond lengths (Å)	0.006	0.005
Bond angles (°)	1.036	0.980

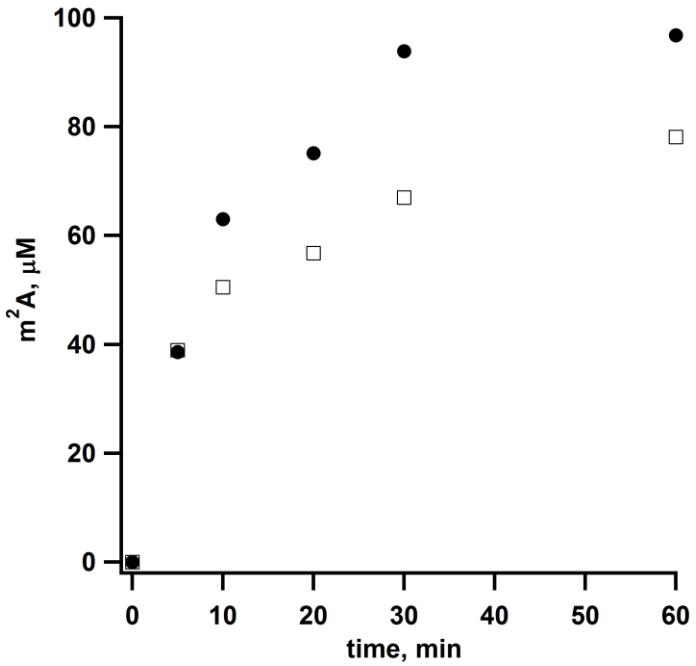

Figure S1. Production of m^2A catalyzed by wt RlmN (black circles) and RlmN E105A (open squares). Assays contained 10 µM wt RlmN or RlmN E105A, 100 µM 155-mer, and 2 mM SAM. Reactions were initiated with addition of 2 mM dithionite.

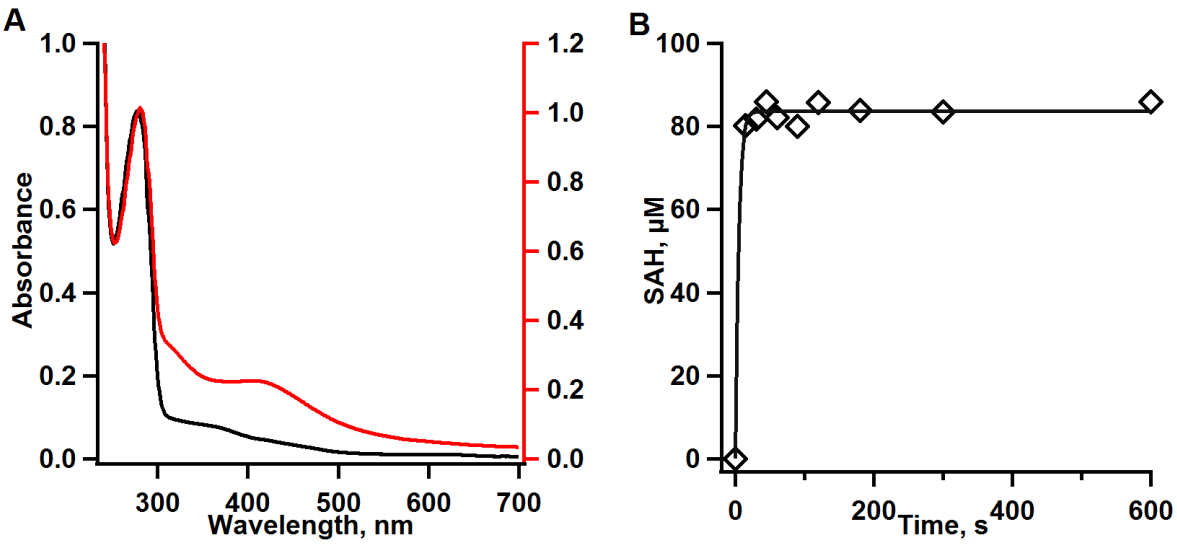

Figure S2: Characterization of the RlmN C118S variant. (A) UV/vis spectrum of apo as-isolated (black, 25 µM) and reconstituted (red, 19 µM) RlmN C118S. (B) SAH formation in the presence of 2 mM SAM and 105 µM apo $RlmN_{C118S \rightarrow RCN}$. The line represents the fit of SAH formation to a pseudo-first order equation, yielding an amplitude of 83 µM SAH.

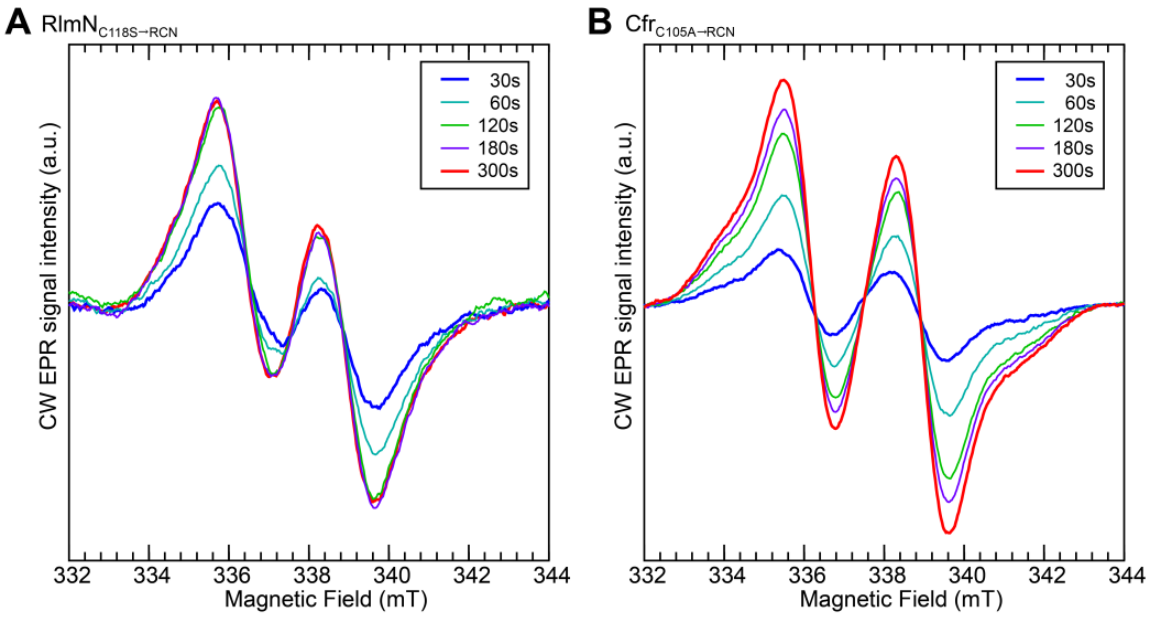

Figure S3: EPR spectra of the cross-linked radical species observed for RlmN$_{C118S→RCN}$ (A) and Cfr$_{C105A→RCN}$ (B) at different times after initiating the reaction (see main text). We note that the shape and the doublet splitting in the EPR spectrum of the Cfr$_{C105A→RCN}$ variant is virtually identical to the one observed in the wt Cfr spectrum, indicating that the C105A amino acid substitution does not substantially perturb the electronic structure and geometry of the cross-linked radical species. Measurements were performed with settings identical those indicated in Figure 2, except that the modulation amplitude was set to 10 G.

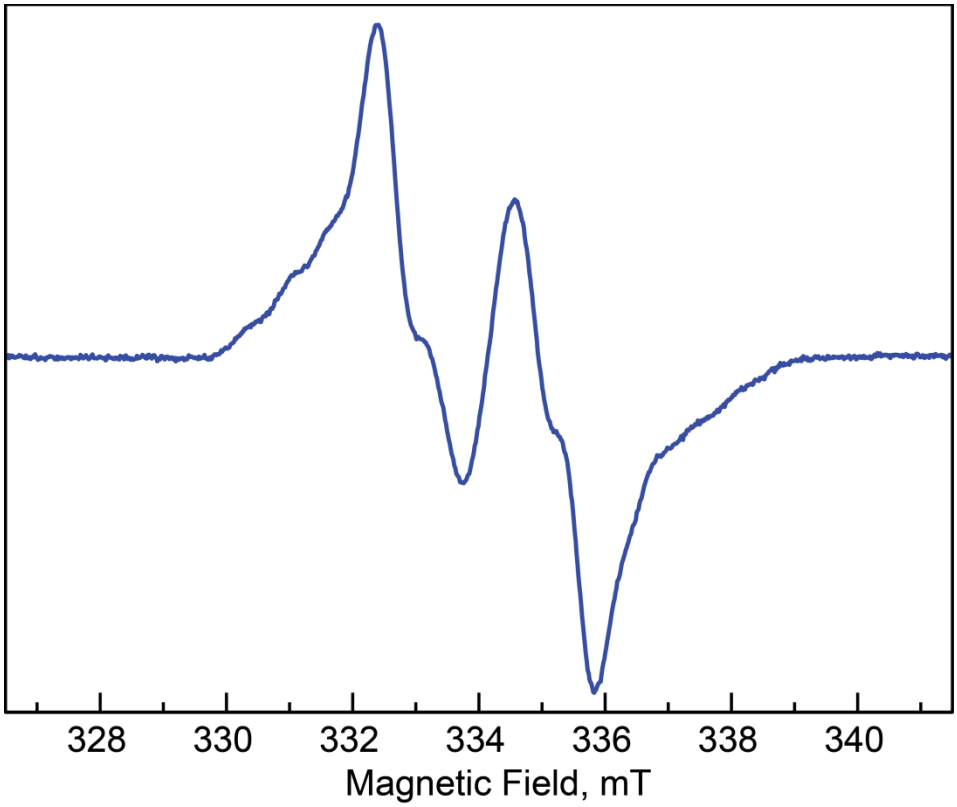

Figure S4. EPR spectrum of the RlmN C118A-155mer cross-linked radical species. Experimental conditions: temperature, 70K; modulation amplitude, 5G; microwave power, 0.12mW; microwave frequency, 9.38 GHz.

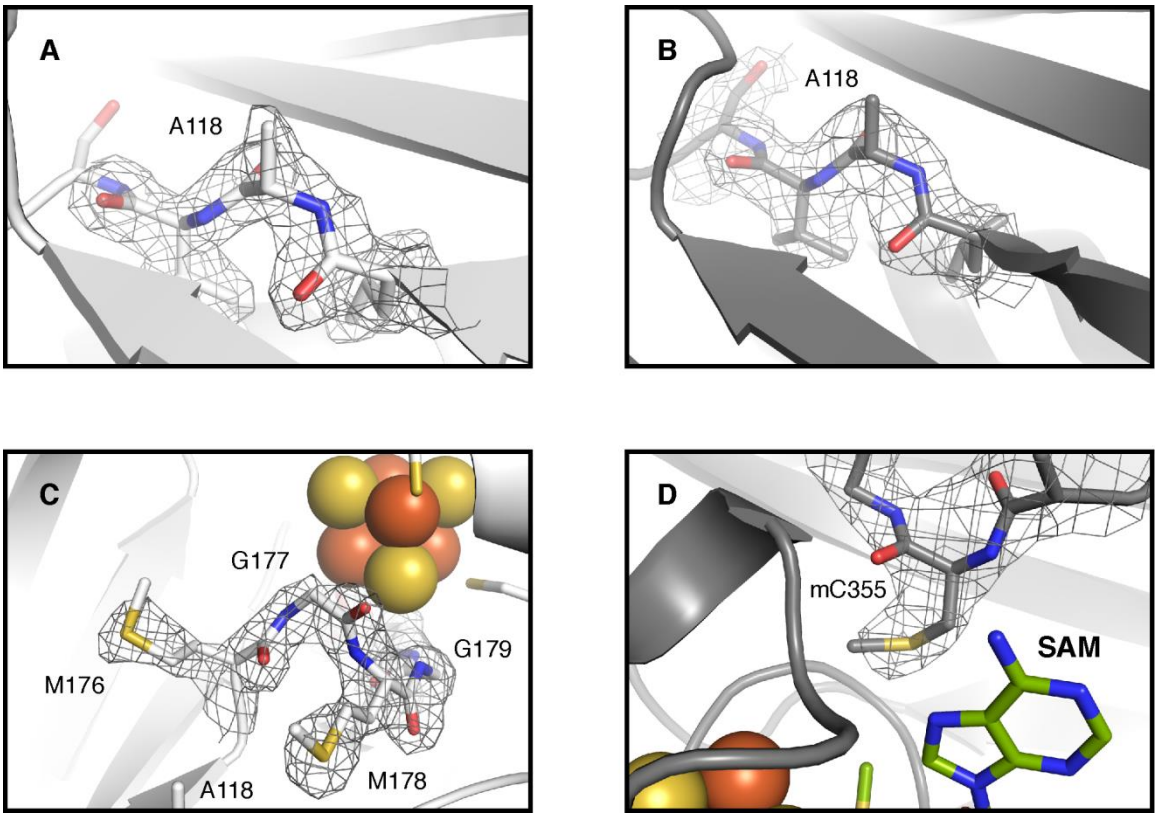

Figure S5. Comparative views of the active site in the C118A RlmN and C118A RlmN + SAM X-ray structures. A $2F_o$-F_c electron density map (gray mesh) for residue 118 and surrounding amino acids in C118A RlmN (**A**) (contoured at 1.5σ) and C118A RlmN + SAM (**B**) (contoured at 1.2σ). A $2F_o$-F_c electron density map is also shown (gray mesh) for the region in C118A RlmN that undergoes a conformational change (residues 176-180) in the absence of SAM (**C**) (contoured at 1.5σ) and for the region near mCys355 in C118A RlmN + SAM (**D**) (contoured at 1.0σ).