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Supporting information for article:

Structure of DnmZ, a nitrososynthase in the *Streptomyces peucetius* anthracycline biosynthetic pathway

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S1. Supplemental Movie Description

DnmZ-ConfChange.mov is a morphing movie that illustrates the conformational differences between the apo-DnmZ and dTDP-DnmZ models. The protein chain is colored grey and shown in cartoon representation. The two regions that undergo significant conformational changes are highlighted in pink. Specific protein residues mentioned in the text are shown in stick representation – D149 and T154 in Loop A; R239, L241 and R243 from Loop B; and E117 and D131, which form hydrogen bonding interactions with R243 and R239 in the dTDP-DnmZ model. The bound dTDP molecule is shown as described in Figure 2a along with modeled FMN and dTDP-3-amino-2,3,6-trideoxy-4-keto-3-methyl-D-glucose (a substrate analog), which are shown in transparent sphere representation. The protein chain morphs between the two models several times and moves between two different camera orientations to afford a clearer view of the structural differences. The animation begins in the same camera orientation as depicted in Figure 4b.

Table S1 Macromolecule source and production information

Source organism	*Streptomyces peucetius*
DNA source	*Streptomyces peucetius* genomic DNA
UniprotKB entry	O33706
Forward primer	CATATGACAAAGCCATCTGTGCACG‡
Reverse primer	AAGCTTTCATCGGCCCAACGCC§
Cloning vector	pET28a†
Expression vector	pET28-ZN
Expression host	*E. coli*
Complete amino acid sequence of the construct produced	MGSSHHHHHHSSGLVPRGSHMTKPSVHEHPGVLADNGLCEPKTP AGRRLLDLLERYLPALEAESRDNDREATLPVHLFDRMRKEGVLG ATVPEDLGGLGVHSLHDVALALARIAGRDAGVALALHMQFSRG LTLDFEWRHGAPSTRPLAEDLLRQMGAGEAVICGAVKDVRGTTV LTRATDGSYRLNGRKTLVSMAGIATHYVVSTRLEEAGAPVRLAA PVVARTTPGLTVLDNWDGMGMRSSGSVDIVFDGCPVDRDRVLP RGEPGVRDDAALAGQTVSSIAMLGIYVGIAEAARRIALTELRRRG GAPAGVRTTVAEIDARLFALHTAVASALTTADRLADDLSGDLAA RGRAMMTPFQYAKLLVNRHSVGVVDDCLMLVGGAGYSNSHPL ARLYRDVRAGGFMHPYNFTDGVDYLSEVALGR
Molecular Weight	45344.6 Da
Biological Functional Unit	Tetramer

‡ The forward primer includes an NdeI restriction site.

§ The reverse primer includes a HindIII restriction site.

† pET28a confers a thrombin-cleavable N-terminal hexahistidine tag on the protein.

Table S2 Protein sequences used to assess sequence conservation among the nitrososynthases.

UniprotKB entry	Annotation	Sequence Identity*	e-value
Q54197_STRGR	Putative oxidoreductase from *Streptomyces griseus*	78	0.0
B5APQ9_9ACTO	Nitrososynthase from *Micromonospora* sp. ATCC 39149 (ORF36)	59	3e-141
D9T1H7_MICAI	Acyl-CoA dehydrogenase domain-containing protein from *Micromonospora aurantiaca*	59	6e-139
K4I7X6_9ACTO	DacS6 from *Dactylosporangium sp. SC14051*	58	2e-138
B3TMR1_9ACTO	KijD3 from *Actinomadura kijaniata*	57	1e-134
B5L6K4_MICCH	Putative flavoprotein OS=*Micromonospora chalcea*	56	4e-132
Q2PC69_STRAH	RubN8 from *Streptomyces achromogenes* subsp. *rubradiris*	56	7e-136
L7RRG4_9ACTO	LobD4 from *Streptomyces sp. FXJ7.023*	55	5e-138
M9T9M4_9ACTO	LobS7 from *Streptomyces* sp. SCSIO 01127	55	1e-137
I2N5E9_9ACTO	Acyl-CoA dehydrogenase domain-containing protein from *Streptomyces tsukubaensis* NRRL18488	55	5e-130

*The sequence identity provided here is that between the enzyme listed and DnmZ.

Table S3 Crystallization Information

Method	Vapor Diffusion
Plate type	Hanging Drop
Temperature (K)	298
Protein concentration	6.0 mg/mL
Buffer composition of protein solution	20 mM Tris-HCl, 1 mM dithiothrethiol, 5% glycerol, pH=7.5
Composition of precipitant solution (apo-DnmZ crystals)	0.1 M glycine, 0.12 M ammonium sulfate, 12% PEG 2000, pH=9.3
Composition of precipitant solution (dTDP-DnmZ crystals)	0.1 M glycine, 0.12 M ammonium sulfate, 12 % PEG 2000, pH 9.4
Volume and ratio of drop	2µL protein:1µL precipitant
Reservoir solution	750µL of 2M ammonium sulfate
Cryoprotectant	None

Table S4 Detailed list of the chain breaks in each DnmZ model.

Model	Chain	Chain Breaks (missing residues)*			
dTDP-DnmZ	A	1-10	159-160	192-193	247-249
	B	1-10	158-163	191-195	247-249
	C	1-10		191	240-242
	D	1-10	160	192-193	
apo-DnmZ	A	1-9		192-193	
	B	1-9		189-193	
	C	1-9	160-162		246-247
	D	1-9		192	

*The chain breaks occur in similar locations in each chain (i.e. at the N-terminus and in surface loops from the central β domain). The missing residues are grouped by their location to emphasize this observation.

Table S5 RMS Deviations between the DnmZ, ORF36 and KijD3 models.

Model	KijD3 with bound flavin and substrate	KijD3 with bound dTDP	ORF36
Apo-DnmZ	0.87± 0.02	0.84± 0.04	0.84± 0.04
dTDP-DnmZ	0.86± 0.04	0.91± 0.03	0.95± 0.04

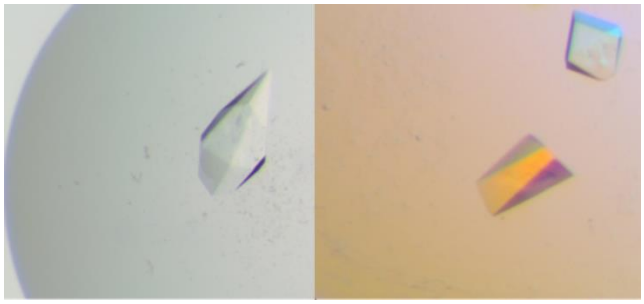

Figure S1 DnmZ crystallizes in a diamond-shaped triclinic form (left) or a square-shaped form that adopts either monoclinic or orthorhombic symmetry (right).

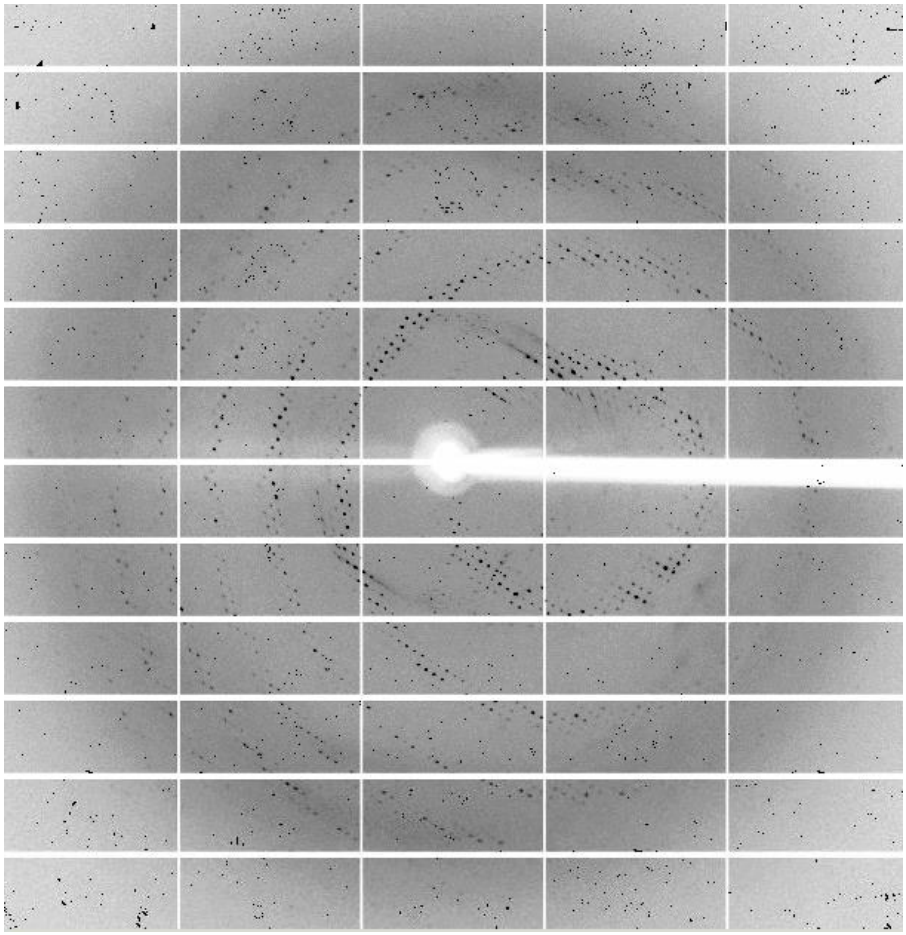

Figure S2 DnmZ diffraction data. This image was part of a dataset collected from orthorhombic DnmZ crystals at SSRL Beamline 12-2.

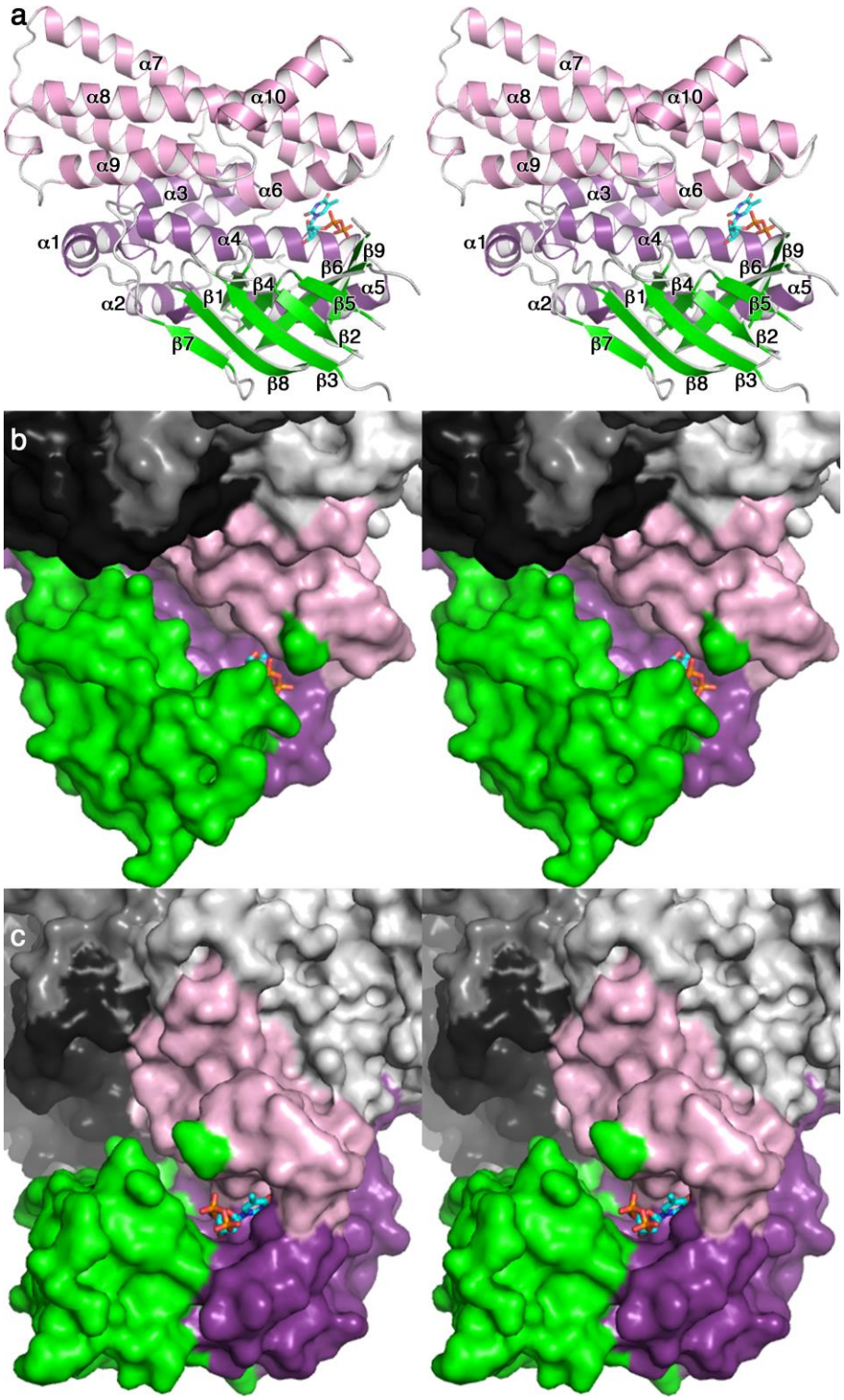

Figure S3 Stereoviews of the DnmZ monomer and active site cleft. (a) The DnmZ monomer is displayed as described in Figure 2b with secondary structural elements labeled. This labeling scheme is consistent with that used to describe the ORF36 structure. dTDP is displayed as thin sticks colored as in Figure 2a. (b) and (c) The dTDP-DnmZ tetramer surface, colored as in Figure 2a with dTDP in stick representation, is shown in two orientations to visualize the active site cleft. In (c) the molecule is rotated 90° with respect to the orientation in (b) about a vertical axis in the plane of the paper.

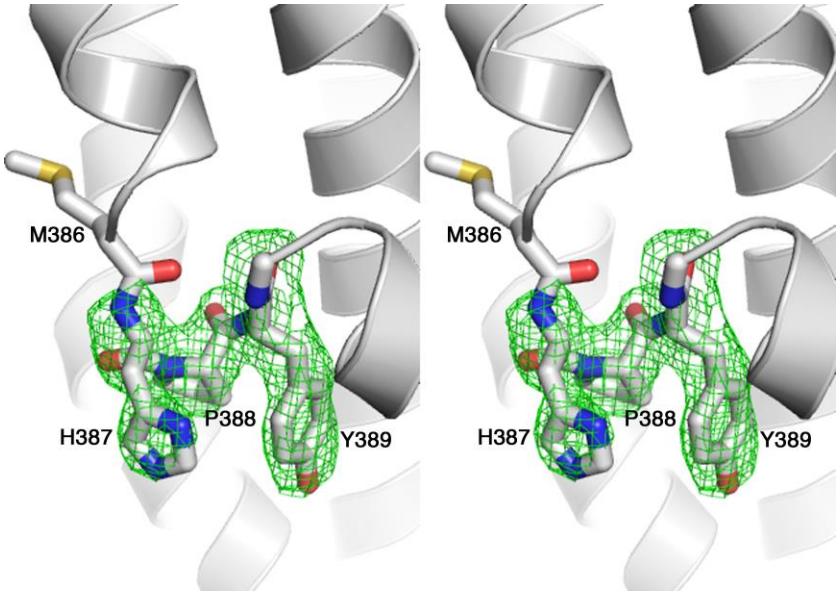

Figure S4 Stereoview of the *cis*-peptide loop with an F_o-F_c omit map contoured at 3.0 sigma shown in green. To generate this figure, residues 387-389 of all four chains were omitted from the model, followed by refinement in REFMAC and recalculation of the electron density maps. The DnmZ protein chain is shown in grey cartoon, with the exception of residues 386-390, which are shown in sticks. The sidechain of N390 is omitted for clarity.

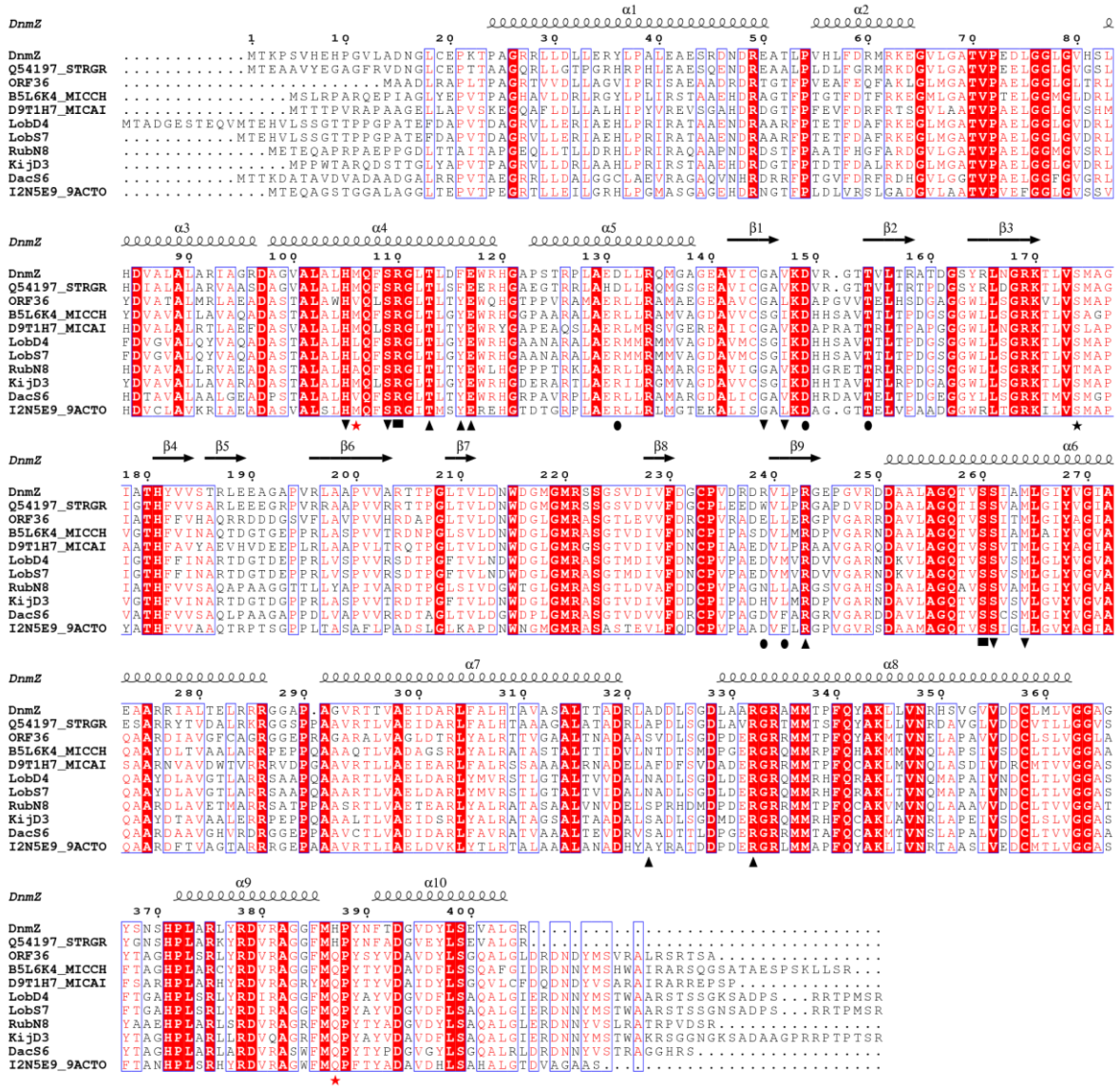

Figure S5 Alignment of selected nitrososynthases. More details on each sequence included in this alignment are summarized in Table S2. The secondary structural elements of DnmZ are shown above the alignment. Specific residues mentioned in the text are identified in the figure as follows: black triangles pointing up, residues involved in interactions with dTDP (T113, F116, E117, R243, A322, R332); black squares, residues that contact the substrate diphosphate group in the KijD3 flavin- and substrate-bound structure (R110, S260); black star, S174; black circles, additional residues noted in the discussion of apo-to-dTDP-bound DnmZ conformational changes (D131, D149, T154, R239, L241, R243); black triangles pointing down, residues in the expected site of catalysis (H105, S109, G145, V147, M204, S261); and red triangles, M106 and H387.

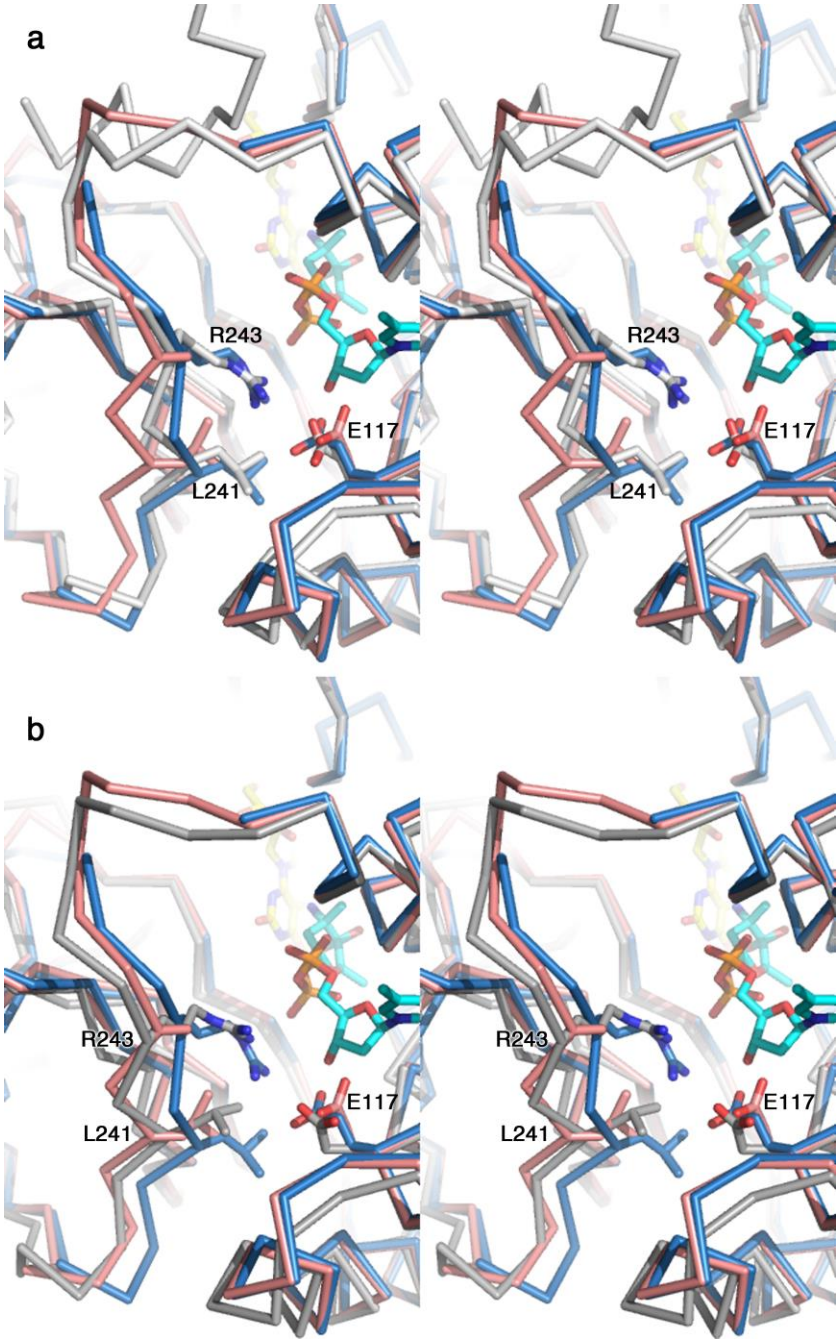

Figure S6 Comparison of apo-DnmZ and dTDP-DnmZ to ORF36 (3MXL, the unliganded enzyme) and KijD3 (4KCF, the flavin and substrate-bound structure) in the Loop B region of the proteins. The proteins are shown in ribbon representation with E117, L241 and R243 shown in sticks. The apo-DnmZ model is shown in pink, the dTDP-DnmZ model is shown in blue, KijD3 (PDB entry 4KCF) is shown in light grey in (a), and ORF36 (PDB entry 3MXL) is shown in dark grey in (b). Both figures are stereoviews.