nature portfolio

https://doi.org/10.1038/s41586-021-03904-6

Supplementary information

Structural basis for tRNA methylthiolation by the radical SAM enzyme MiaB

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Supplementary Information for

"Structural basis for tRNA methylthiolation by the radical SAM enzyme MiaB"

Authors: Olga A. Esakova[1,*], Tyler L. Grove[2,*], Neela H. Yennawar[4], Arthur J. Arcinas[3,6], Bo Wang[1], Carsten Krebs[1,3], Steven C. Almo[2], and Squire J. Booker[1,3,5*]

Affiliations: The [1]Department of Chemistry, the [3]Department of Biochemistry and Molecular Biology, [4]The Huck Institutes of the Life Sciences, and the [5]Howard Hughes Medical Institute, The Pennsylvania State University, University Park, Pennsylvania 16802, USA.

The [2]Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York

[6]Present address: AGC Biologics, Seattle, WA

Corresponding authors: squire@psu.edu, oae3@psu.edu, and tylergrove223@gmail.com

Table of Contents

Table S1: The root mean square deviation (RMSD) for each model against the structure of *Bu*MiaB with a pentasulfide was calculated using The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC

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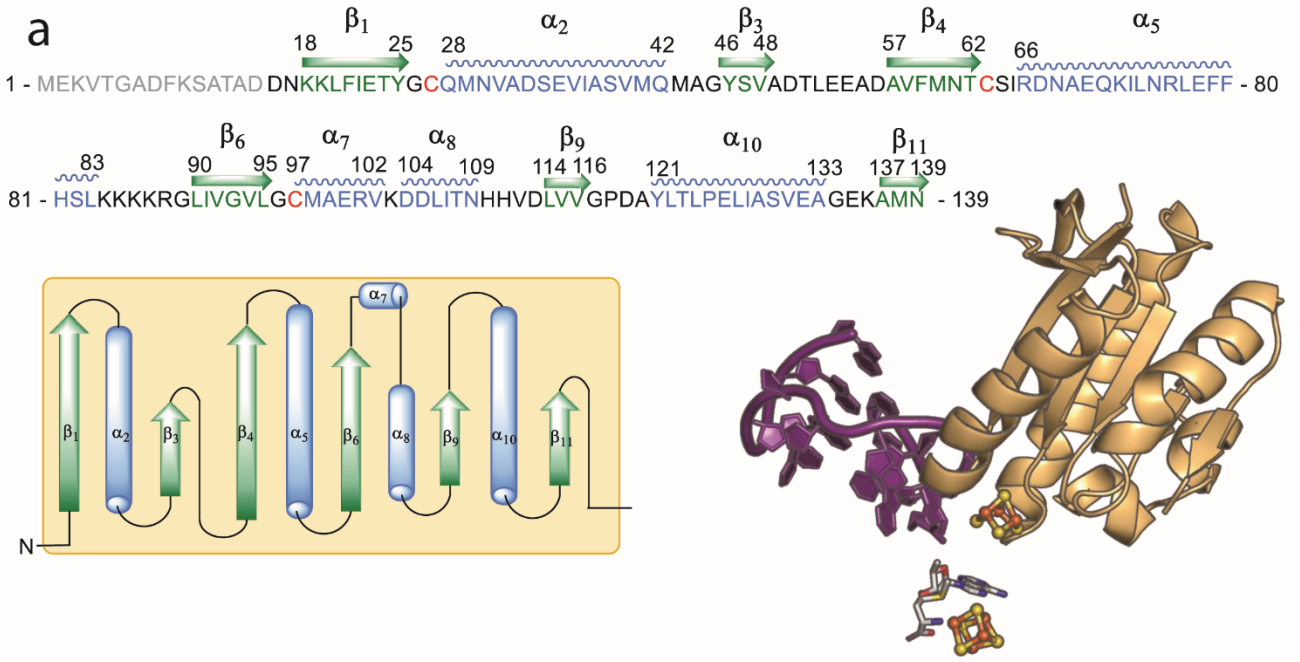
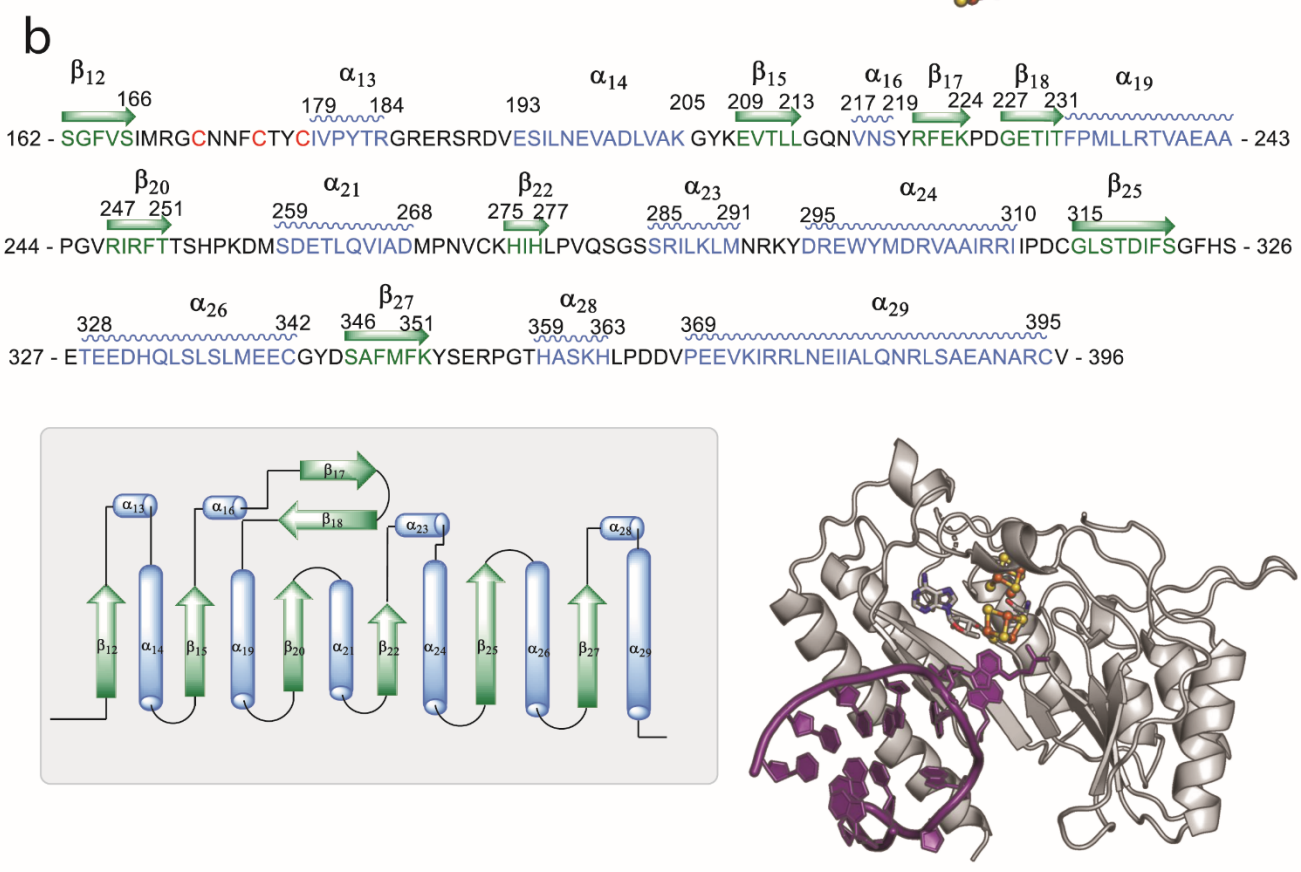
Structure	PDB entry	Chain	RMSD (Å) *Main chain*
Bacteroides uniformis MiaB with pentasulfide bridge	7MJZ	A	reference
Thermatoga maritima RimO	4JC0	A B	1.6 1.5
Bacteroides uniformis MiaB co-crystallized with SAH and 13-mer ACSL $tRNA^{Phe}$	7MJY	A	0.4
Bacteroides uniformis MiaB co-crystallized with SAM and 17-mer ACSL $tRNA^{Phe}$	7MJV	A	0.4
Bacteroides uniformis MiaB co-crystallized with 5'dAH+Met and 13-mer ACSL $tRNA^{Phe}$	7MJX	A B	0.9 1.0
Methylated *Bacteroides uniformis* MiaB co-crystallized with 5'dAH+Met and 13-mer ACSL $tRNA^{Phe}$	7MJW	A B	1.0 0.9


a
β1 18 25
α2 28 42
β3 46 48
β4 57 62
α5 66
1 - MEKVTGADFKSATAD DNKKLFIETYGCQMNVADSEVIASVMQ MAGYSVADTLEEADAVFMNTCSIRDNAEQKILNRLEFF - 80
β6 83 90 95
α7 97 102
α8 104 109
β9 114 116
α10 121 133
β11 137 139
81 - HSLKKKKRGLIVGVLGCMAERVKDDLITNHHVDLVVGPDAYLTLPELIASVEAGEKAMN - 139
α7
β1 α2 β3 β4 α5 β6 α8 β9 α10 β11
N


b
β12 166
α13 179 184
α14 193
β15 205 209 213
α16 217 219
β17 224
β18 227 231
α19
162 - SGFVSIMRGCNNFCTYCIVPYTRGRERSRDVESILNEVADLVAK GYKEVTLLGQNVNSYRFEKPDGETITFPMLLRTVAEAA - 243
β20 247 251
α21 259 268
β22 275 277
α23 285 291
α24 295 310
β25 315
244 - PGVRIRFTTSHPKDMSDETLQVIADMPNVCKHIHLPVQSGSSRILKLMNRKYDREWYMDRVAAIRRIIPDCGLSTDIFSGFHS - 326
α26 328 342
β27 346 351
α28 359 363
α29 369 395
327 - ETEEDHQLSLSLMEECGYDSAFMFKYSERPGTHASKHLPDDVPEEVKIRRLNEIIALQNRLSAEANARCV - 396
β17
α13
α16
β18
α23
α28
β12 α14 β15 α19 β20 α21 β22 α24 β25 α26 β27 α29

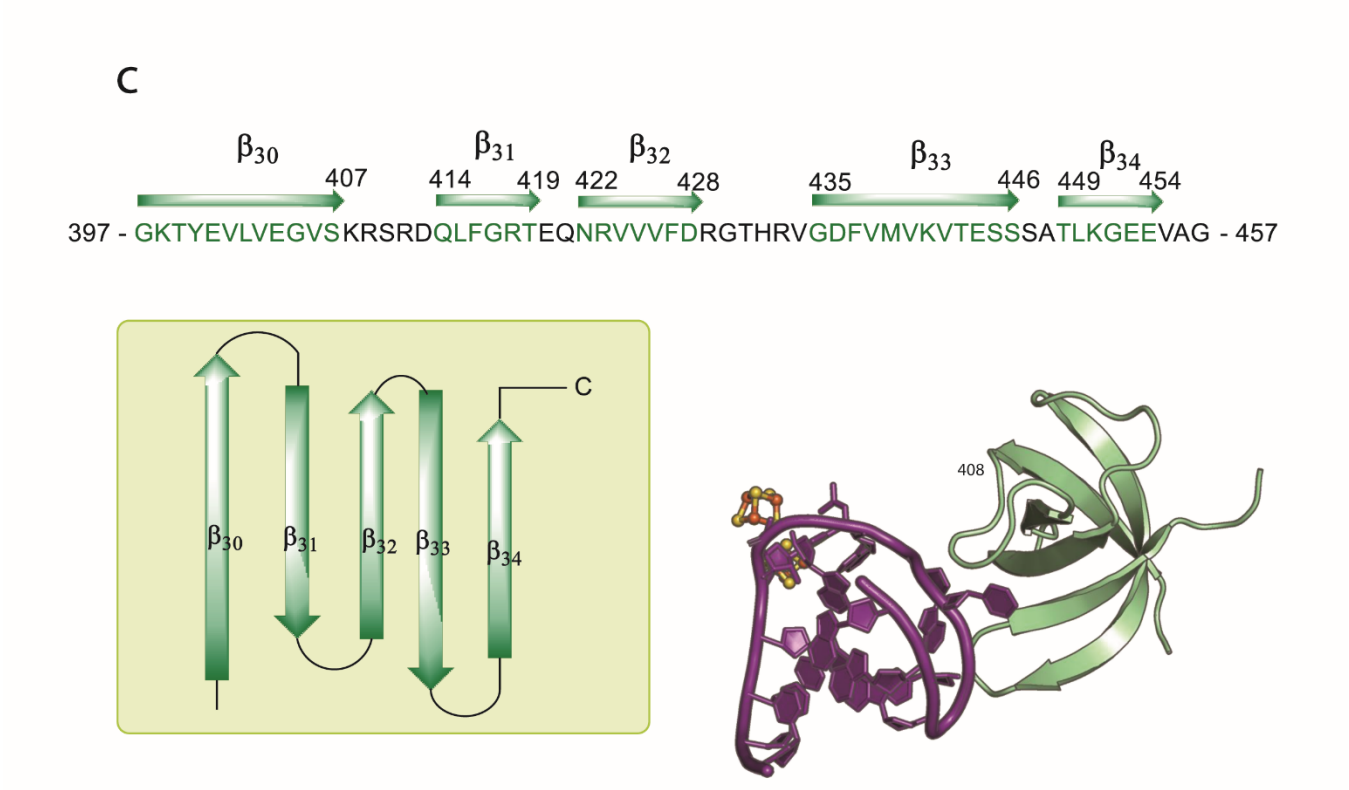


Fig. S1. Sequence, secondary structure schematic, and cartoon of the three-dimensional fold of *Bu*MiaB. **a**, N-terminal MTTase domain. **b**, radical SAM domain. **c**, C-terminal TRAM domain. Beta sheets and alpha-helices in the schematics are represented in green and blue colors respectively. The radical SAM domain exhibits a conserved core fold that consists of a shortened $(\beta\alpha)_6$ triosephosphate isomerase (TIM) barrel, and contains four short alpha helical extensions (α13, α16, α23 and α28) that make interactions with the cluster binding loop (residues from 167 to 178), as well as a solvent-exposed two-stranded β-sheet (β17- β18). All figures have the same color scheme for the domains and their associated residues: tan for MTTase, gray for radical SAM and green for TRAM. The cysteine residues involved in coordination of the clusters are colored in red.

a.

DNA

ATGGAAAAAGTTACCGGTGCCGATTTCAAAAGCGCAACCGCAGATGATAACAAAAAGCTGTTTATTGAAAC
CTACGGCTGCCAGATGAATGTTGCAGATAGCGAAGTTATTGCAAGCGTTATGCAGATGGCAGGTTATAGCG
TTGCGGATACCCTGGAAGAGGCAGATGCAGTTTTTATGAATACCTGTAGCATTCGCGATAACGCCGAACAG
AAAATTCTGAATCGTCTGGAATTTTTCCACAGCCTGAAAAAAAAGAAACGCGGTCTGATTGTTGGTGTTCT
GGGTTGTATGGCAGAACGTGTTAAAGATGATCTGATCACCAATCATCATGTGGATCTGGTTGTTGGTCCGG
ATGCATATCTGACCCTGCCGGAACTGATTGCCAGCGTTGAAGCCGGTGAAAAAGCAATGAATGTGGAACTG
AGCACCACCGAAACCTATCGTGATGTTATTCCGAGCCGTATTTGCGGTAATCATATTAGCGGTTTTGTGAG
CATTATGCGTGGCTGTAATAACTTTTGCACCTATTGCATTGTTCCGTATACACGTGGTCGTGAACGTAGCC
GTGATGTTGAAAGCATTCTGAATGAAGTTGCCGATCTGGTGGCAAAAGGTTATAAAGAAGTTACCCTGCTG
GGTCAGAATGTGAATAGCTATCGTTTTGAAAAACCGGATGGTGAAACCATTACCTTTCCGATGCTGCTGCG
TACCGTTGCCGAAGCAGCACCGGGTGTTCGTATTCGTTTTACCACCAGTCATCCGAAAGATATGAGTGATG
AAACCCTGCAGGTTATTGCAGATATGCCGAATGTGTGCAAACATATTCATCTGCCGGTTCAGAGCGGTAGC
AGCCGTATTCTGAAACTGATGAATCGTAAATATGATCGCGAGTGGTATATGGATCGTGTTGCAGCAATTCG
TCGTATTATTCCGGATTGTGGTCTGAGCACCGATATCTTTAGTGGTTTTCATAGCGAAACCGAAGAAGATC
ATCAGCTGAGCCTGAGTCTGATGGAAGAATGTGGTTATGATAGCGCCTTCATGTTCAAATATAGCGAACGT
CCGGGTACACATGCAAGCAAACATCTGCCTGATGATGTTCCGGAAGAAGTTAAAATTCGTCGCCTGAATGA
AATTATTGCCCTGCAGAATCGCCTGAGTGCGGAAGCAAATGCACGTTGTGTTGGTAAAACCTATGAAGTTC
TGGTTGAAGGTGTTAGCAAACGTTCACGTGATCAGCTGTTTGGTCGTACAGAACAGAATCGTGTTGTTGTT
TTTGATCGTGGCACCCATCGTGTGGGTGATTTTGTTATGGTTAAAGTGACCGAAAGCAGCAGCGCAACCCT
GAAAGGTGAAGAAGTTGCAGGTTAA

b.

Protein:

MEKVTGADFKSATADDNKKLFIETYGCQMNVADSEVIASVMQMAGYSVADTLEEADAVFMNTCSIRDNAEQKILNRLE
FFHSLKKKKRGLIVGVLGCMAERVKDDLITNHHVDLVVGPDAYLTLPELIASVEAGEKAMNVELSTTETYRDVIPSRI
CGNHISGFVSIMRGCNNFCTYCIVPYTRGRERSRDVESILNEVADLVAKGYKEVTLLGQNVNSYRFEKPDGETITFPM
LLRTVAEAAPGVRIRFTTSHPKDMSDETLQVIADMPNVCKHIHLPVQSGSSRILKLMNRKYDREWYMDRVAAIRRIIP
DCGLSTDIFSGFHSETEEDHQLSLSLMEECGYDSAFMFKYSERPGTHASKHLPDDVPEEVKIRRLNEIIALQNRLSAE
ANARCVGKTYEVLVEGVSKRSRDQLFGRTEQNRVVVFDRGTHRVGDFVMVKVTESSSATLKGEEVAG*

Fig. S2. *Bu*MiaB DNA and protein sequences for structures with substrates. a, DNA sequence. **b**, Protein sequence. Note that His363 (shown in red) is Y363 in the native *Bu*MiaB structure