

1 Supplemental Material

2 Identification of a unique radical SAM methylase likely involved in methanopterin biosynthesis
3 in *Methanocaldococcus jannaschii*

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10 Running head: Radical SAM methylase in methanopterin biosynthesis

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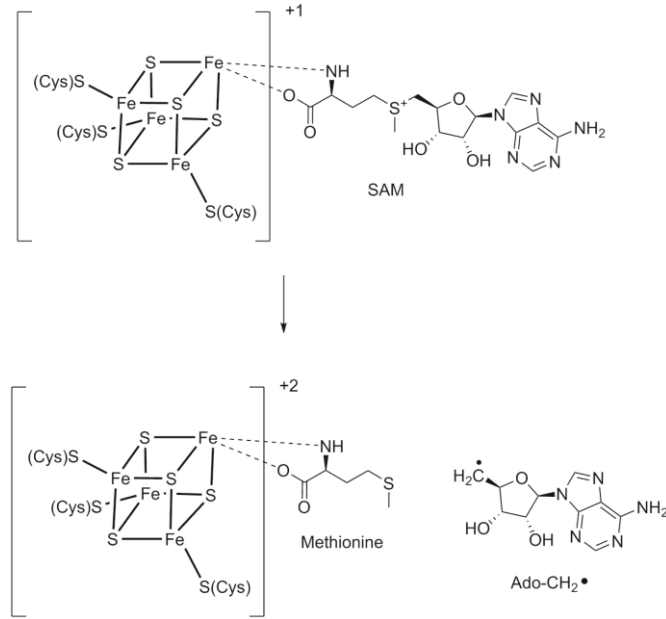
Compound	Precursor ion (m/z)	Product ion (m/z)	Dwell time (ms)	DP (V)	CE (V)
7-methylpterin	178.1	133.1	100	20	33
	178.1	106.1	100	20	33
	178.1	92.1	75	20	33
6-ethyl-7-methylpterin	206.1	179.1	100	20	30
	206.1	163.1	100	20	30
6-hydroxyethyl-7-methylpterin	222.1	204.2	100	20	30
	222.1	161.1	100	20	30
	222.1	132.1	75	20	30
Folate	440.3	311.1	100	-55	-30
Folate + 14*	454.3	325.3	100	-55	-30
Folate + 28**	468.3	339.3	100	-55	-30

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21 Supplemental Table 1. Multiple reaction monitoring (MRM) mode parameters for LC-ESI-MS
22 detection of pterins and folates in *E. coli* cell extracts.

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24 *Folate with one methyl group at C-7 or C-9.
25 **Folate with two methyl groups at C-7 and C-9 or a formyl group. The product ions cannot
26 distinguish between the two possibilities. Since both the *E.coli*_MJ0619 and the *E.coli*_control
27 cells have this peak, we concluded that the observed peak is *N*¹⁰-formylfolate.

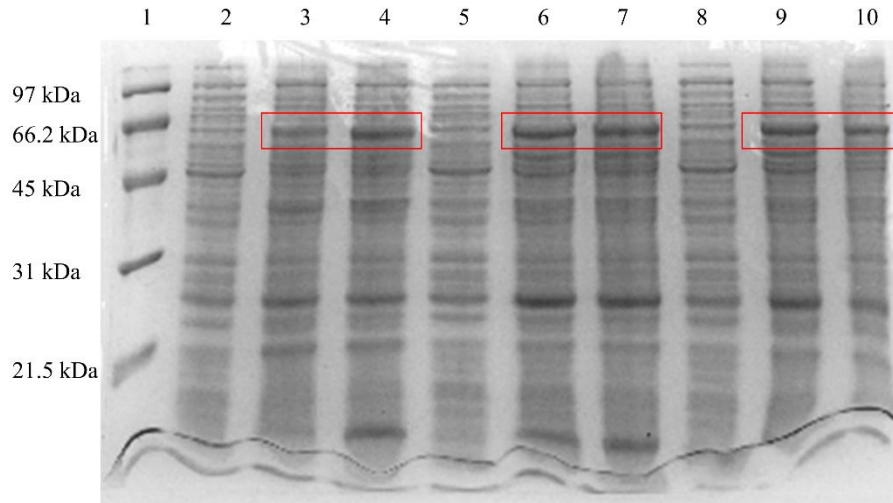
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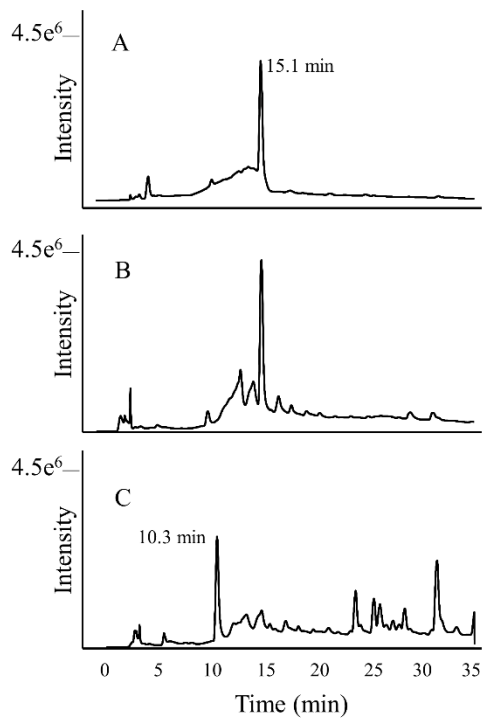
Supplemental Figure 1. First step in radical SAM enzyme catalysis to form the 5'-deoxyadenosyl radical (Ado-CH₂•).

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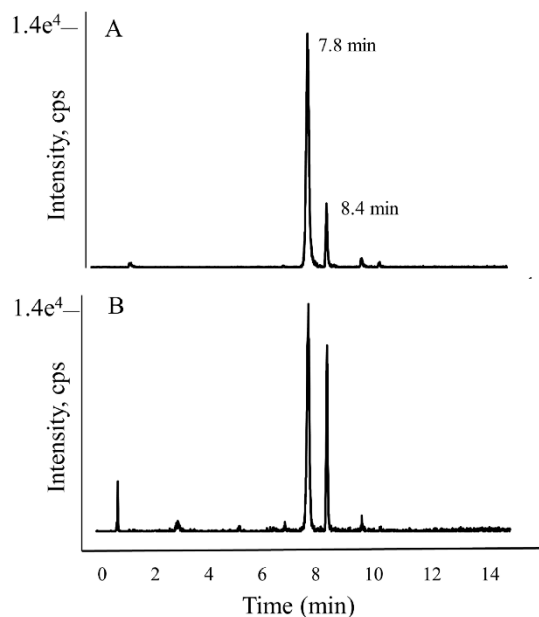
Supplemental Figure 2. SDS-PAGE gel of MJ0619, MJ0619-M1, and MJ0619-M2 expression in *E. coli* grown in LB media. Lane 1- Molecular weight marker, Lane 2- *E. coli* + pMJ0619 before induction, Lane 3- *E. coli* + pMJ0619 2 h after induction, Lane 4- *E. coli* + pMJ0619 4 h after induction, Lane 5- *E. coli* + pMJ0619-M1 before induction, Lane 6- *E. coli* + pMJ0619-M1 2 h after induction, Lane 7- *E. coli* + pMJ0619-M1 4 h after induction, Lane 8- *E. coli* + pMJ0619-M2 before induction, Lane 9- *E. coli* + pMJ0619-M2 2 h after induction, Lane 10- *E. coli* + pMJ0619-M2 4 h after induction. The red boxes highlight the MJ0619 protein band. Protein identity was confirmed by MALDI mass spectral analysis.

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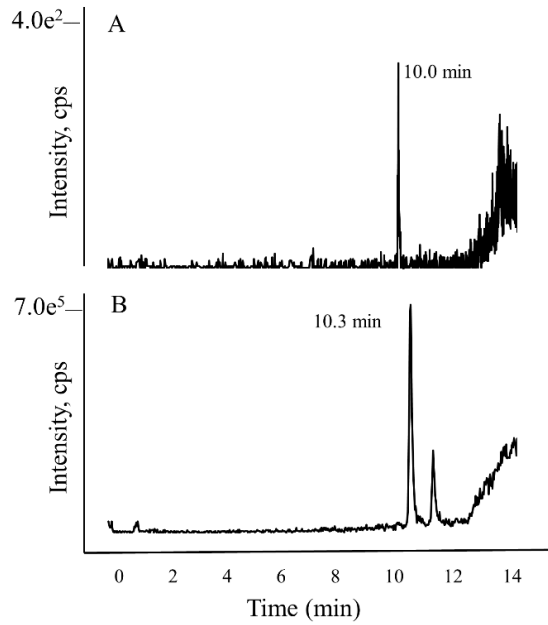
Supplemental Figure 3. HPLC fluorescence chromatogram of extracted and partially purified pterins. (A) authentic 7-methylpterin, (B) pterins from *E. coli*_MJ0619 and, (C) pterins from *E. coli*_control. 10.3 min peak is pterin.

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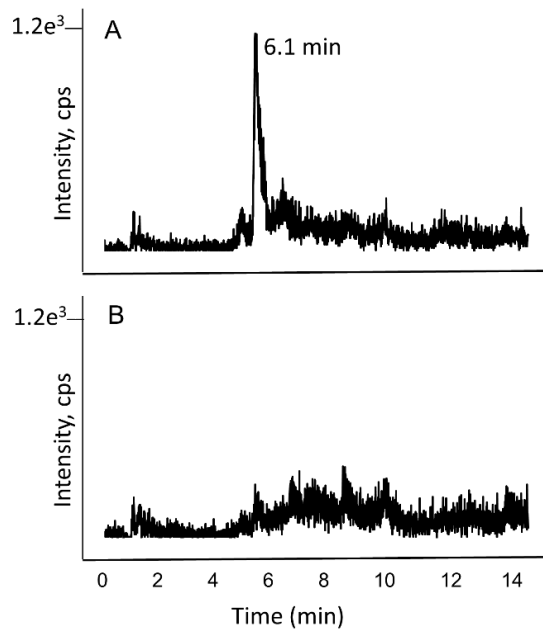
Supplemental Figure 4. LC-MS MRM ion chromatogram of extracted and partially purified folates from *E. coli* cells. (A) Folates isolated from *E.coli_MJ0619* cells and (B) Folates isolated from *E.coli_control* cells. 7.8 min peak is N^{10} -formylfolate and 8.4 min peak is folate.

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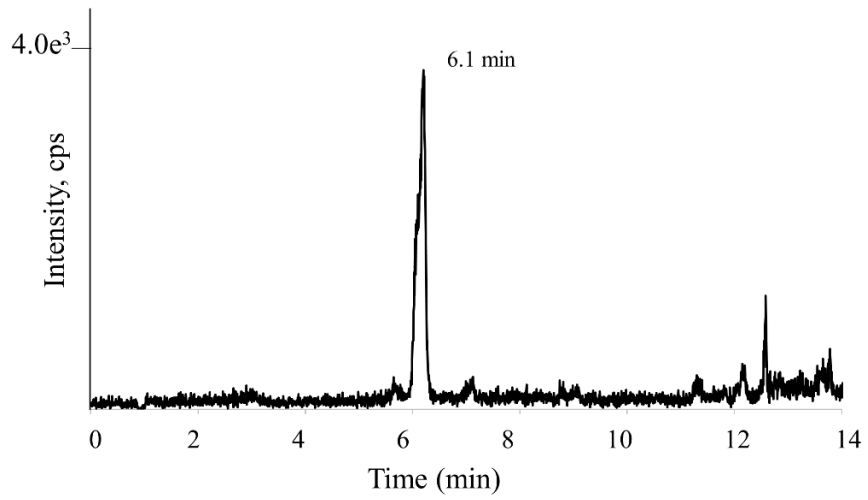
Supplemental Figure 5. LC-MS MRM ion chromatogram of synthetic methylated folates. (A) 7-methylfolate and (B) 7, 9- dimethylfolate (10.3 min).

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Supplemental Figure 6. LC-MS MRM traces for 7-methylpterin from *E. coli*_MJ0619 Cys to Ala variants. (A) MJ0619 C77A and (B) MJ0619 C102A. The peak at 6.1 min corresponds to 7-methylpterin.

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Supplemental Figure 7. LC-MS MRM trace for 7-methylpterin from *E. coli*_MJ0619 grown in M9 media, demonstrating that the enzyme does not require cobalamin for methylation activity. The peak at 6.1 min corresponds to 7-methylpterin.