Supplemental Material

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

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Running head: Radical SAM methylase in methanopterin biosynthesis

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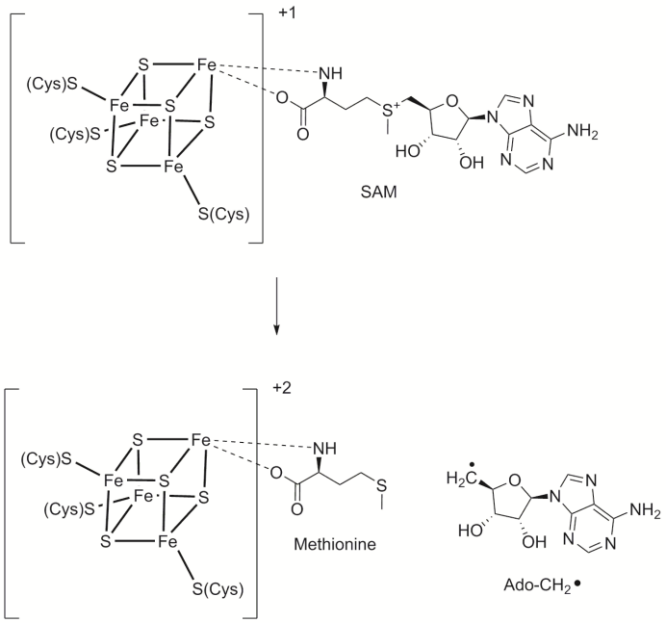
Compound	**Precursor ion *(m/z)***	**Product ion *(m/z)***	**Dwell time (ms)**	**DP (V)**	**CE (V)**
7-methylpterin	178.1	133.1	100	20	33
	178.1	106.1	100	20	33
	178.1	92.1	75	20	33
6-ethyl-7-methylpterin	206.1	179.1	100	20	30
	206.1	163.1	100	20	30
6-hydroxyethyl-7-methylpterin	222.1	204.2	100	20	30
	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

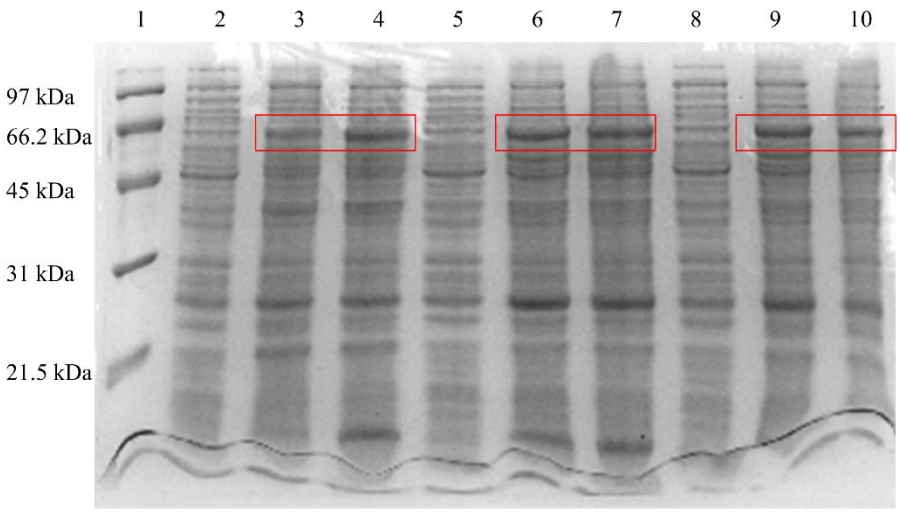
Supplemental Table 1. Multiple reaction monitoring (MRM) mode parameters for LC-ESI-MS detection of pterins and folates in *E. coli* cell extracts.

*Folate with one methyl group at C-7 or C-9.

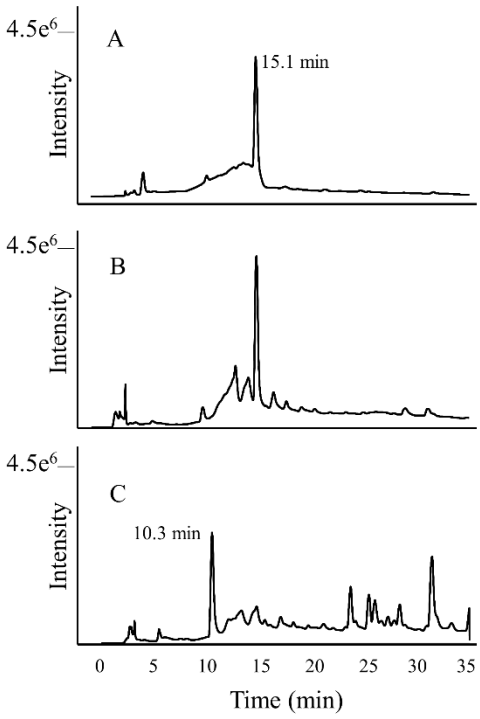
**Folate with two methyl groups at C-7 and C-9 or a formyl group. The product ions cannot distinguish between the two possibilities. Since both the *E.coli*_MJ0619 and the *E.coli*_control cells have this peak, we concluded that the observed peak is N^{10}-formylfolate.



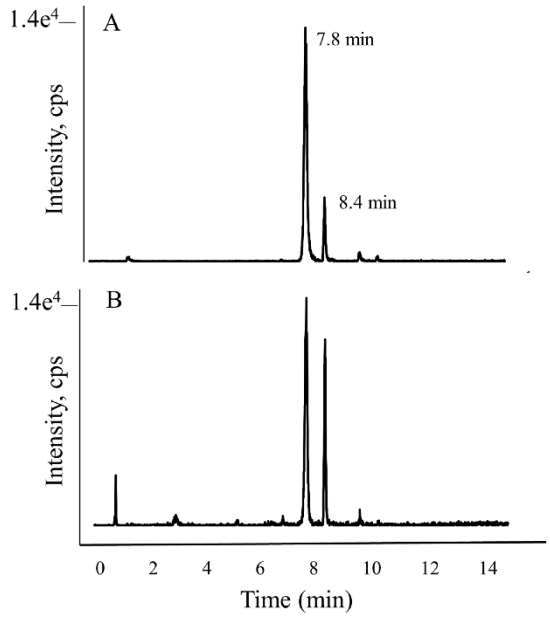
Supplemental Figure 1. First step in radical SAM enzyme catalysis to form the 5′-deoxyadenosyl radical (Ado-CH_2•).



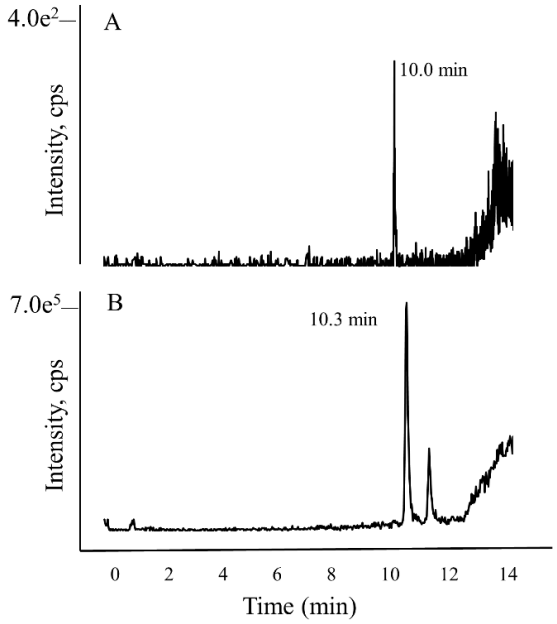
Supplemental Figure 2. SDS-PAGE gel of MJ0619, MJ0619-M1, and MJ0619-M2 expression in *E. coli* grown in LB media. Lane 1- Molecular weight marker, Lane 2- *E. coli* + pMJ0619 before induction, Lane 3- *E. coli* + pMJ0619 2 h after induction, Lane 4- *E. coli* + pMJ0619 4 h after induction, Lane 5- *E. 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.



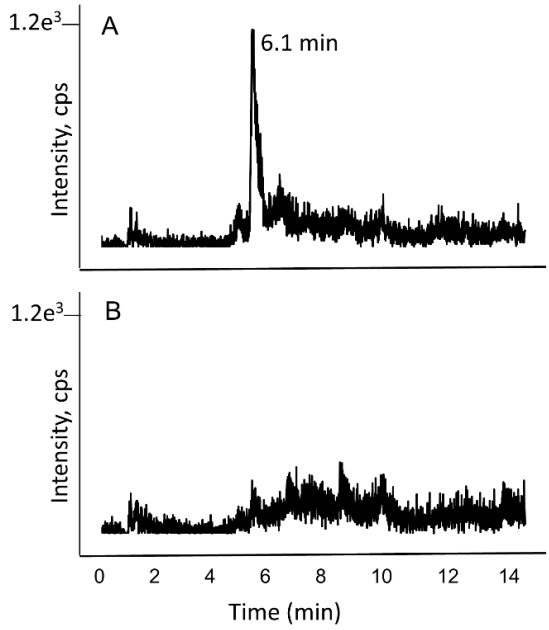
Supplemental Figure 3. HPLC fluorescence chromatogram of extracted and partially purified pterins. (A) authentic 7-methylpterin, (B) pterins from *E. coli*_MJ0619 and, (C) pterins from *E. coli*_control. 10.3 min peak is pterin.



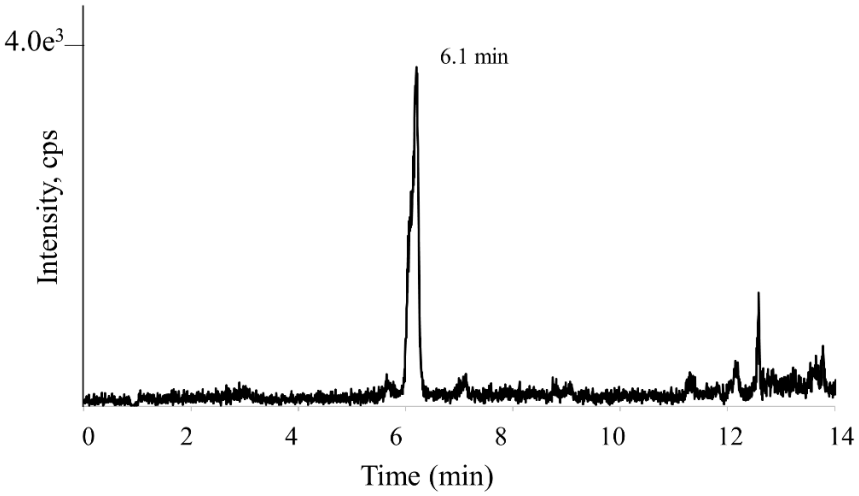
Supplemental Figure 4. LC-MS MRM ion chromatogram of extracted and partially purified folates from *E. coli* cells. (A) Folates isolated from *E.coli*_MJ0619 cells and (B) Folates isolated from *E.coli*_control cells. 7.8 min peak is N^{10}-formylfolate and 8.4 min peak is folate.



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



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.



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.