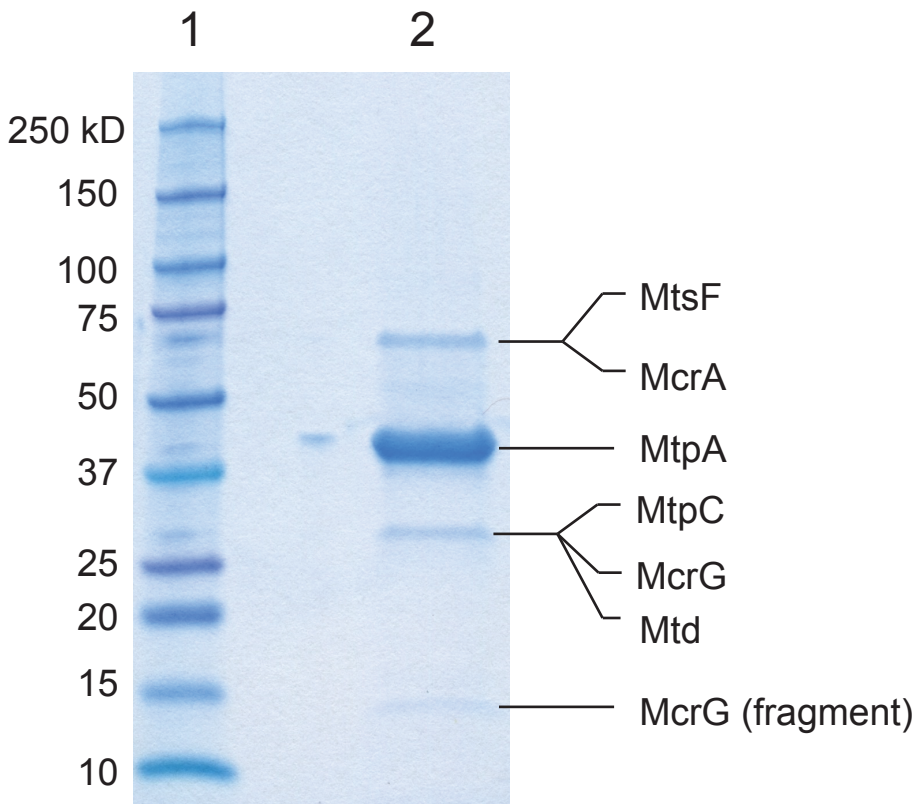

Figure S1. SDS-PAGE analysis of affinity purified MtpA from a Δ*mtpA* host. Cell-free extract from WWM903 was subjected to affinity purification. The eluted proteins were then separated by SDS-PAGE electrophoresis (4-20% gradient gel) and stained with Coomassie blue. While MtpA was the predominant protein, multiple additional proteins were observed. Lane 1: Molecular weight markers (molecular masses are indicated in kDa to the left). Lane 2: proteins eluted from Strep-Tactin resin. Proteins identified by mass-spectrometry as described in the Methods section are indicated. Full dataset can be found in Supplementary dataset 1.

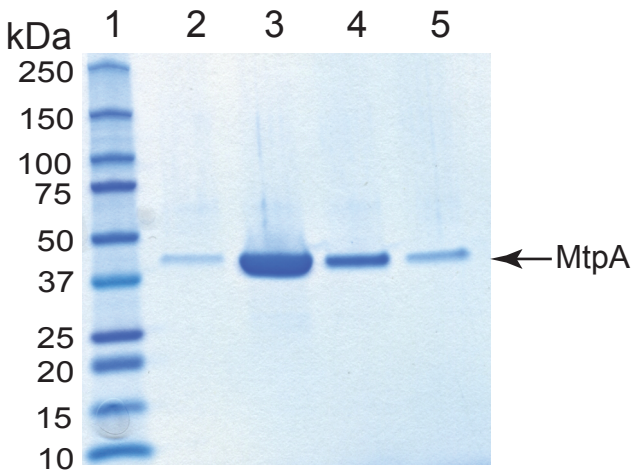

Figure S2. SDS-PAGE analysis of affinity purified MtpA from a Δ*mtsD,* Δ*mtsF,* Δ*mtsH,* Δ*mtpCAP* host. Cell-free extract from WWM998 was subjected to affinity purification. The eluted proteins were then separated by SDS-PAGE (4-20%) electrophoresis and stained with Coomassie Blue. Lane 1: Molecular weight markers (masses are indicated in kDa to the left); Lanes 2-5: elution fractions of purified MtpA. The predicted size for strep-tagged MtpA is 38.7 kDa.

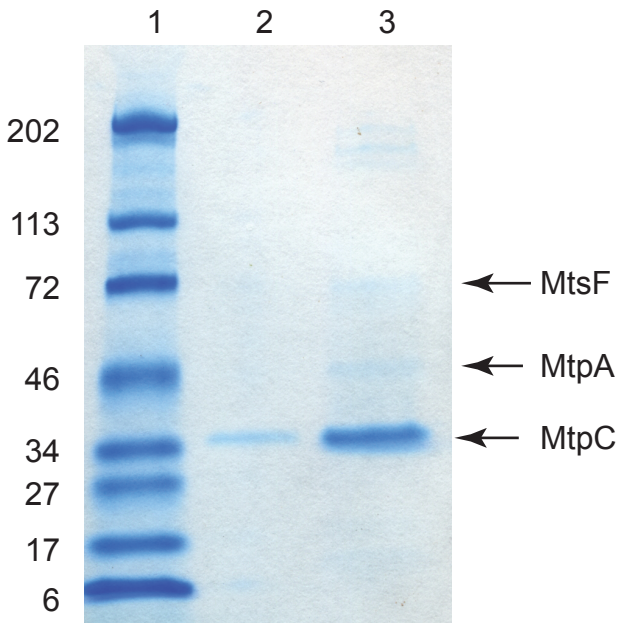

Figure S3. SDS-PAGE analysis of affinity purified MtpC. Cell-free extract from WWM902 was subjected to affinity purification. The eluted proteins separated by SDS-PAGE electrophoresis (4-20% gradient gel) and stained with Coomassie blue. While MtpC was the predominant protein, multiple additional proteins were observed. Lane 1: Molecular weight markers (molecular masses are indicated in kDa to the left). Lane 2: proteins eluted from Strep-Tactin resin. Lane 3: 25-fold concentration of eluted proteins. Proteins identified by mass-spectrometry are indicated.

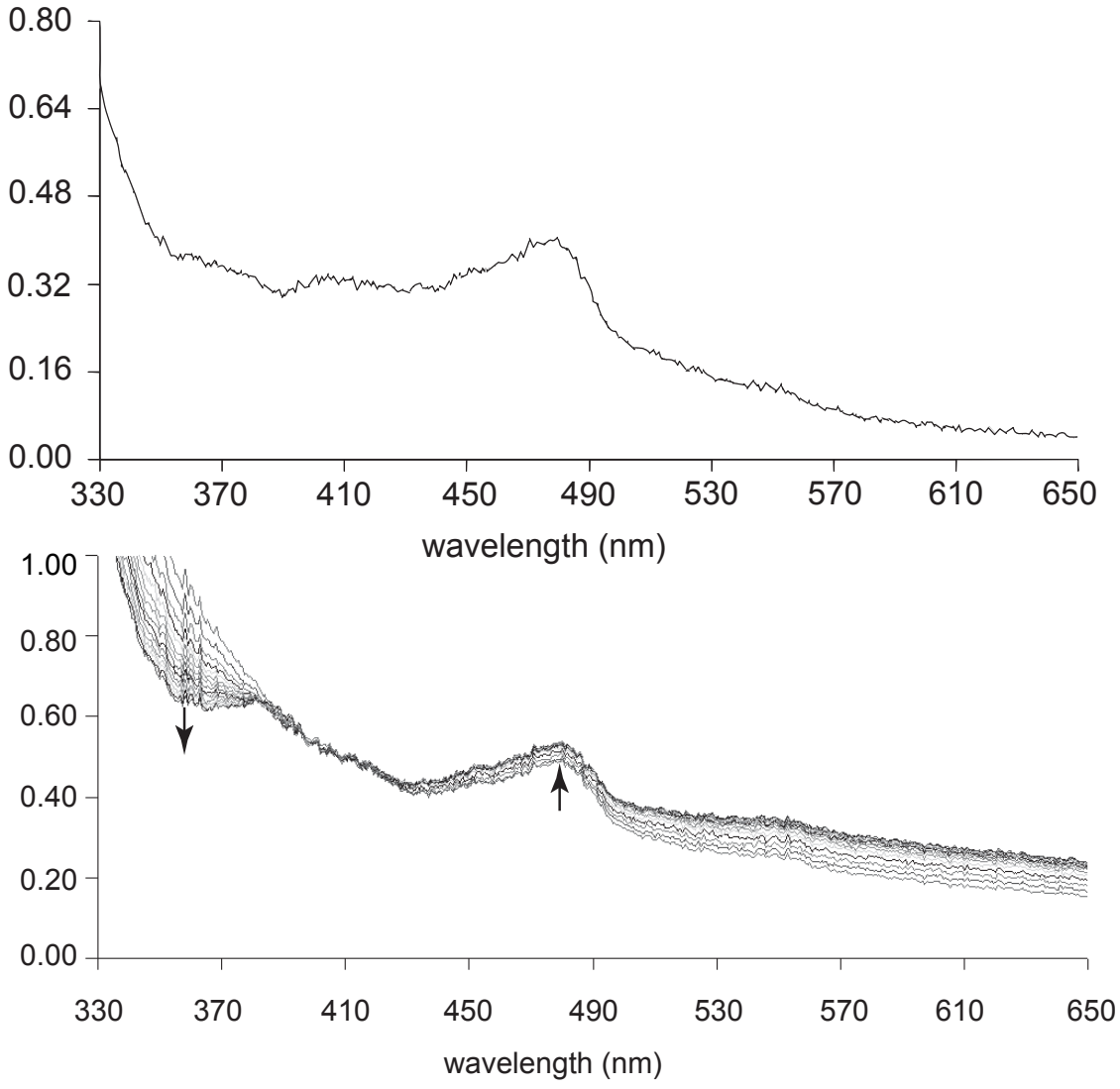

Figure S4. The B12HBI cofactor in purified the MtpC/MtpA complex is in the inactive Co(II) state and cannot be reactivated. *Upper panel,* The UV-visible spectrum of the as-isolated MtpC/MtpA complex. The diagnostic peak at 475 nm, the trough at 385 nm, and shoulder at 540 nm are characteristic of cobalamin at Co(II) state. *Lower panel,* The UV-visible spectrum of the MtpC/MtpA complex Incubation of the MtpC/MtpA complex during a 15-hour incubation with Ti(III) citrate/methyl viologen. Diagnostic Co(I) peaks at 388 and 540 nm were not observed. Note that the spectrum is slightly different than that in the upper panel due to the presence of Ti(III) citrate/methyl viologen.

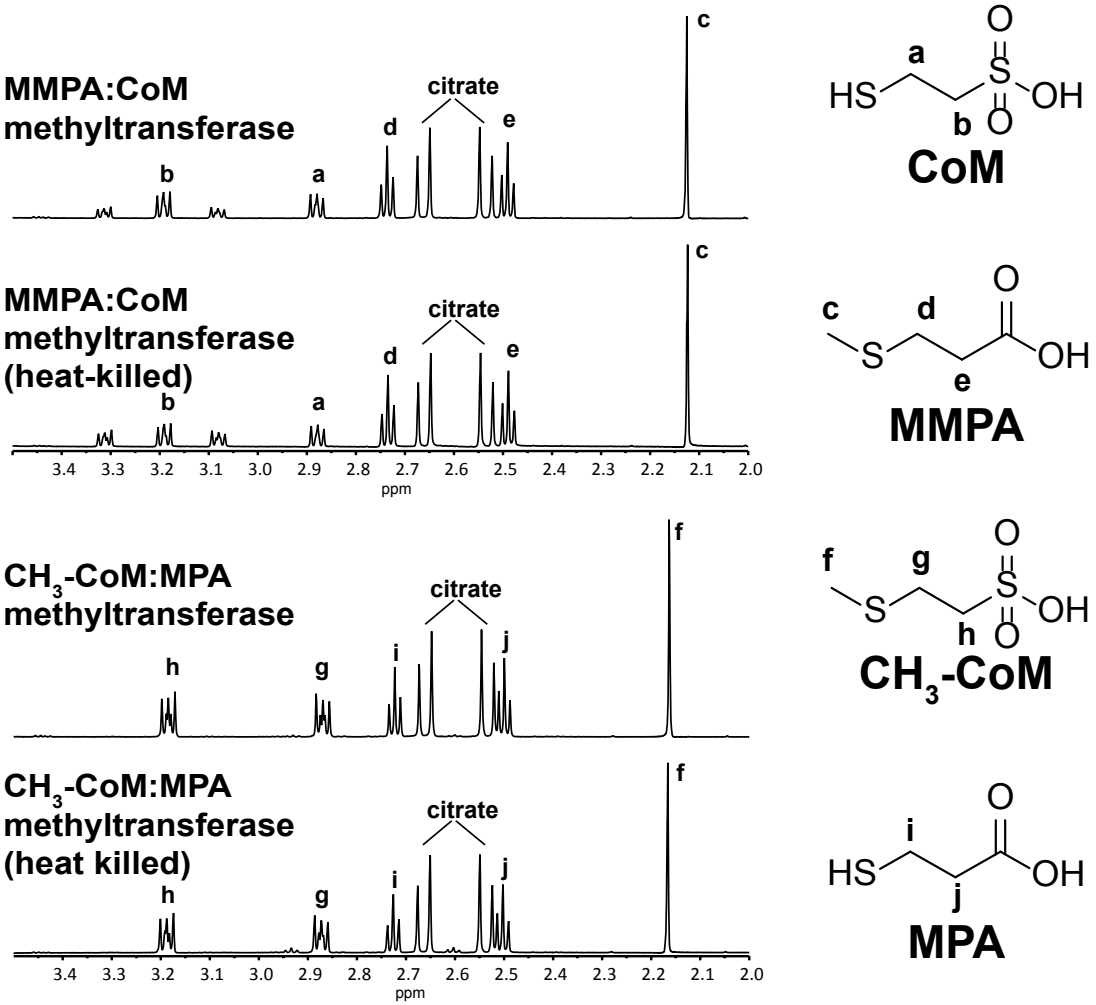

Figure S5. ^{1}H-NMR assay of MtpC/MtpA methyltransferase activity. The MMPA:CoM methyltransferase assays included CoM, MMPA and the "reactivated" MtpC/MtpA complex. Positive activity would have resulted in the production of CH_3-CoM and MPA. The CH_3-CoM:MPA methyltransferase assays included CH_3-CoM, MPA and the "reactivated" MtpC/MtpA complex. Positive activity would have resulted in the production of CoM and MMPA. Assays were incubated for 16 hours prior to collection of NMR spectra. The chemical shifts for each proton are labeled a-j in the NMR spectra and the chemical structures of substrates and products shown on the right. Protons attributable to citrate from the Ti(III)-citrate in the reactivation mix are also shown in the NMR spectra. The MtpC/MtpA complex was boiled for 5 min prior to addition for the heat-killed reactions.

Table S1. Plasmids used in the study

Plasmid	Description	Source
pJK026A	λ-attB, ϕC31-attB vector with PmcrB promoter fusion to uidA	(1)
pJK027A	λ-attB, ϕC31-attB vector with PmcrB(tetO1) promoter fusion to uidA	(1)
pAMG40	λ-attP, *E.coli-Methanosarcina* shuttle vector for retrofitting plasmids	(1)
pFH018	λ-attB, ϕC31-attB vector with PmcrB(tetO1) promoter fusion to C-terminus strep-tagged *mtpC*	This study
pFH020	λ-attB, ϕC31-attB vector with PmcrB(tetO1) promoter fusion to C-terminus strep-tagged *mtpA*	This study
pFH035	λ-attB, ϕC31-attB vector with PmcrB promoter fusion to C-terminus strep-tagged *mtpC* and C-terminus strep-tagged *mtpA*	This study
pFH036	Fusion plasmid of pAMG40 and pFH035	This study
pFH041	Fusion plasmid of pAMG40 and pFH018	This study
pFH042	Fusion plasmid of pAMG40 and pFH020	This study

Table S2. Primers used in the study

Name	**Sequence**	**Added site**
C-tag_MA4164_for	GGCGCGCCCATATGGATGACCTACCGGAAGAAGTCCAG	NdeI
C-tag_MA4164_rev	GGCGCGCCTGCAGGTCATTTTTCAAACTGAGGGTGGGACCAACTCCATCTCACGCTTTCAGACGG	SbfI
C-tag_MA4165_for	GGCGCGCCCATATGGTATCTGAGATGACCTCAAAAGAAAGGGTC	NdeI
C-tag_MA4165_rev	GGCGCGCCTGCAGGTCATTTTTCAAACTGAGGGTGGGACCAAAGCTTATCTGCAGACCAGTCAGCTG	SbfI
mtpC_tag_rev	GTAAAGTTCATTTTTCAAACTGAGGGTGGGACCAACTCCATCTCACGCTTTCAGACGG	none
mtpA_for	GAGATGGAGTTGGTCCCACCCTCAGTTTGAAAAATGAACTTTACAGGTGTTTTTGCG	none
mtpA-F02	GGCGCGCCCTCGAGATGGTATCTGAGATGACCTCAAAAGAAAG	XhoI
mtpA-R02	GGCGCGCCGGATCCCAAAGCTTATCTGATCAAAGCTTATCTGC	BamHI
N-tag_MA4164_for	GGCGCGCCCATATGTGGTCCCACCCTCAGTTTGAAAAAGATGACCTACCGGAAGAAGTCCAG	NdeI
N-tag_MA4164_rev	GGCGCGCCTGCAGGCTGTAAAGTTCAACTCCATCTCACGCTTTC	none
N-tag_MA4165_for	GGCGCGCCCATATGTGGTCCCACCCTCAGTTTGAAAAAATGGTATCTGAGATGACCTCAAAAGAAAGG	NdeI
N-tag_MA4165_rev	GGCGCGCCTGCAGGACCAAAGCTTATCTGATCAAAGCTTATCTGC	none

1. **Guss AM, Rother M, Zhang JK, Kulkarni G, Metcalf WW.** 2008. New methods for tightly regulated gene expression and highly efficient chromosomal integration of cloned genes for Methanosarcina species. Archaea **2:**193-203.