Supporting Information for:

Methanogenesis marker protein 10 (Mmp10) from *Methanosarcina acetivorans* is a radical *S*-adenosylmethionine methylase that unexpectedly requires cobalamin

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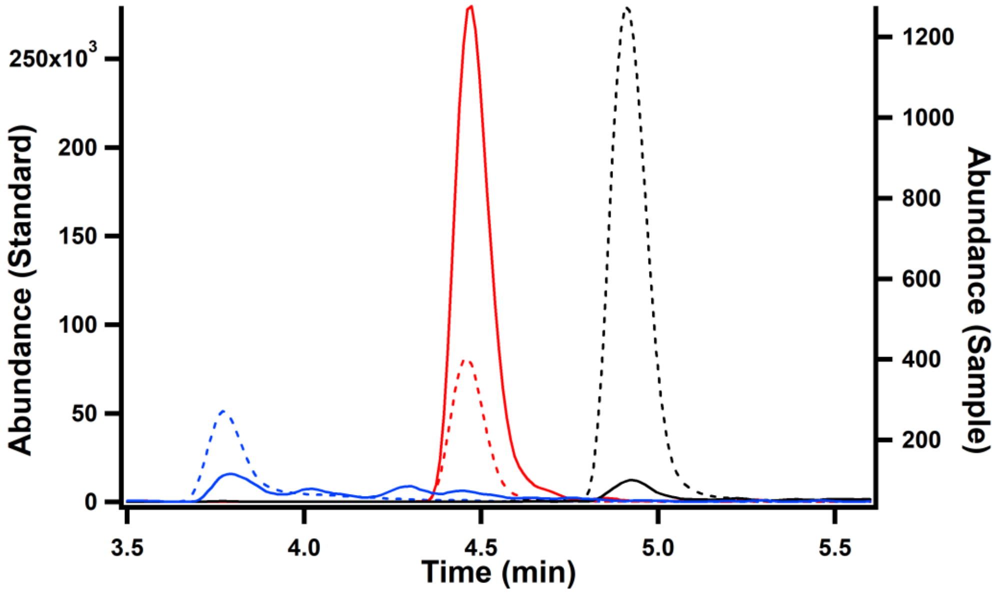


Figure S1. LC-MS analysis of cobalamin species bound to 1560 µM *as-purified Ma*Mmp10. *Ma*Mmp10-associated OHCbl (solid blue line), *Ma*Mmp10-associated AdoCbl (solid red line), *Ma*Mmp10-associated MeCbl (solid black line), OHCbl standard (dashed blue line), AdoCbl standard (dashed red line), MeCbl standard (dashed black line). *m/z* transition for OHCbl, 664.9 → 635.8 $[M+2H]^{2+}$; MeCbl, 673.0 → 665.3 $[M+2H]^{2+}$; AdoCbl 790.6 → 665.3 $[M+2H]^{2+}$.

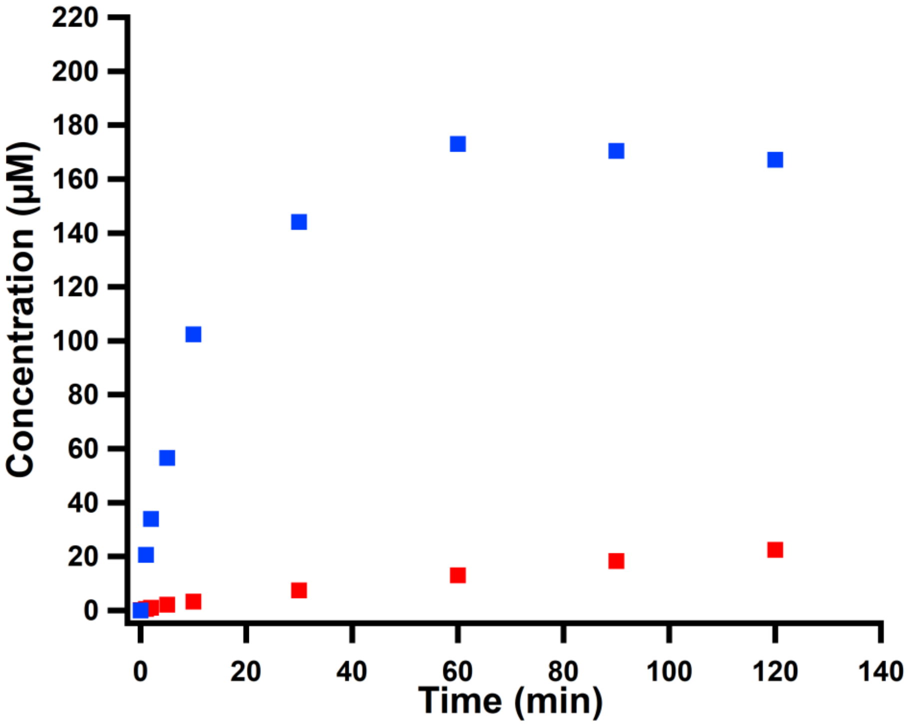


Figure S2. **Activity of *as-purified Ma*Mmp10 versus Cbl-Rc *Ma*Mmp10.** Concentration of methylated peptide product for *Ma*Mmp10 reaction (red squares) and concentration of methylated peptide product from Cbl-Rc *Ma*Mmp10 reaction (blue squares). Reactions were conducted at 30 °C and contained the following components in a final volume of 200 µL: 50 mM HEPES, pH 7.5, 200 mM KCl, 50 µM *Ma*Mmp10, 280 µM peptide substrate, and 1 mM Ti(III) citrate. Reactions were initiated with 1 mM SAM.

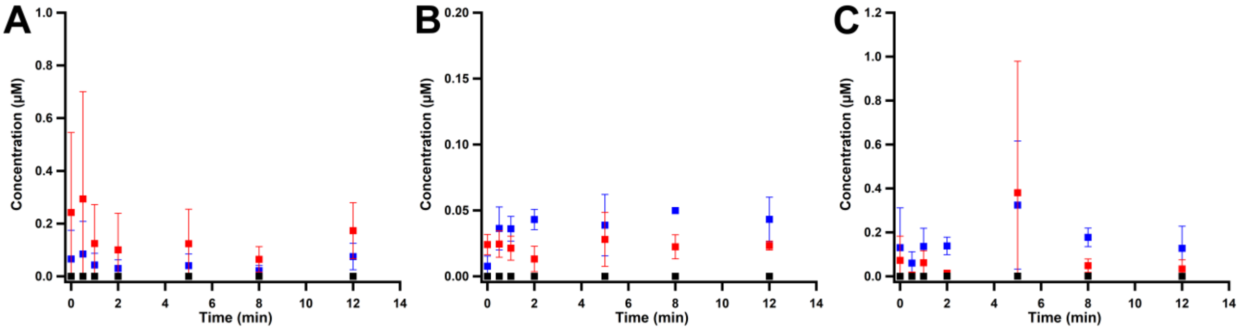
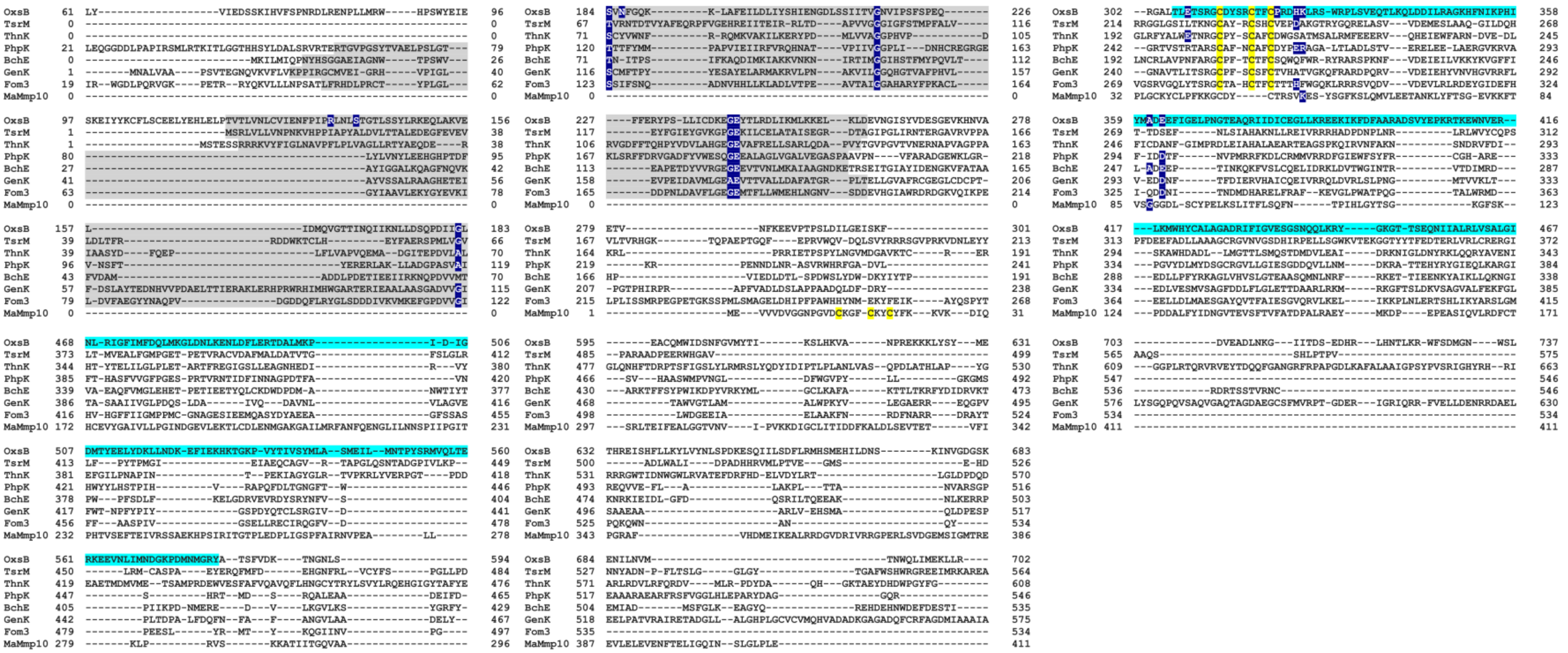


Figure S3. 5′-dAH, SAH, and methylated peptide formation in the absence of SAM, in the absence of Ti(III) citrate, or in the absence of *Ma*Mmp10.

(A) Cbl-Rc *Ma*Mmp10 activity in the absence of SAM. (B) Cbl-Rc *Ma*Mmp10 activity in the absence of Ti(III) citrate. (C) Formation of reaction products in the absence of Cbl-Rc *Ma*Mmp10. For Panels A-C, 5′-dAH is indicated by red squares, SAH by blue squares, and methylated peptide product by black squares. Reactions were performed in triplicate at 30 ˚C, and contained the following components in a final volume of 180 µL unless noted otherwise: 50 mM HEPES, 200 mM KCl, 10 µM *Ma*Mmp10, 0.5 mM Ti(III) citrate, and 100 µM peptide substrate. Reactions were initiated with 0.5 mM SAM or an equivalent volume of buffer.



```
OxsB      61 LY-------------------VIEDSSKIHVFSPNRDLRENPLLMRW-----HPSWYEIE  96
TsrM       0 ------------------------------------------------------------  0
ThnK       0 ------------------------------------------------------------  0
PhpK      21 LEQGGDDLPAPIRSMLRTKITLGGTHHSYLDALSRVRTERTGVPGSYTVAELPSLGT---  79
BchE       0 --------------------------MKILMIQPNYHSGGAEIAGNW-----TPSWV---  26
GenK       1 -------MNALVAA----PSVTEGNQVKVFLVKPPIRGCMVEI-GRH-----VPIGL---  40
Fom3      19 IR--WGDLPQRVGK----PETR--RYQKVLLLNPSATLFRHDLPRCT-----YPLGL---  62
MaMmp10    0 ------------------------------------------------------------  0

OxsB      97 SKEIYYKCFLSCEELYEHLELPTVTLVNLCVIENFPIPRLNLSTGTLSSYLRKEQLAKVE  156
TsrM       1 ----------------------MSRLVLLVNPNKVHPPIAPYALDVLTTALEDEGFEVEV  38
ThnK       1 ------------------MSTESSRRRKVYFIGLNAVPFLPLVAGLLRTYAEQDE----R  38
PhpK      80 --------------------------------------------LYLVNYLEEHGHPTDF  95
BchE      27 --------------------------------------------AYIGGALKQAGFNQVK  42
GenK      41 --------------------------------------------AYVSSALRAAGHETEI  56
Fom3      63 --------------------------------------------GYIAAVLEKYGYEVKI  78
MaMmp10    0 ------------------------------------------------------------  0

OxsB     157 L---------------------------------IDMQVGTTINQIIKNLLDSQPDIIGL  183
TsrM      39 LDLTFR-----------------------RDDWKTCLH---------EYFAERSPMLVGV  66
ThnK      39 IAASYD----FQEP----------------------LFLVAPVQEMA--DGITEPDVLAL  70
PhpK      96 V-NSFT----------------------------------YERERLAK-LLADGPASVAI  119
BchE      43 FVDAM--------------------------------ADDLPDETIEEIIRKNQPDVVMT  70
GenK      57 F-DSLAYTEDNHVVPDAELTTIERAKLERHPRWRHIMHWGARTERIEAALAASGADVVGI  115
Fom3      79 L-DVFAEGYYNAQPV---------------DGDDQFLRYGLSDDDIVKVMKEFGPDVVGI  122
MaMmp10    0 ------------------------------------------------------------  0

OxsB     468 NL-RIGFIMFDQLMKGLDNLKENLDFLERTDALMKP------------------I-D-IG  506
TsrM     373 LT-MVEALFGMPGET-PETVRACVDAFMALDATVTG------------------FSLGLR  412
ThnK     344 HT-YTELILGLPLET-ARTFREGIGSLLEAGNHEDI------------------R---VY  380
PhpK     385 FT-HASFVVGFPGES-PRTVRNTIDFINNAGPDTFA----------------------VN  420
BchE     339 VA-EAQFVMGLEHET-PETIEETYQLCKDWDPDM-A------------------NWTIYT  377
GenK     386 TA-SAAIIVGLPDQS-LDA------IVQ---DAVNL------------------VLAGVE  416
Fom3     416 HV-HGFFIIGMPPMC-GNAGESIEEMQASYDYAEEA------------------GFSSAS  455
MaMmp10  172 HCEVYGAIVLLPGINDGEVLEKTLCDLENMGAKGAILMRFANFQENGLILNNSPIIPGIT  231

OxsB     507 DMTYEELYDKLLNDK-EFIEKHKTGKP-VYTIVSYMLA--SMEIL--MNTPYSRMVQLTE  560
TsrM     413 LF---PYTPMGI--------------EIAEQCAGV--R--TAPGLQSNTADGPIVLKP--  449
ThnK     381 EFGILPNAPIN--------------T--PEKIAGYGLR--TVPKRLYVERPGT----PDD  418
PhpK     421 HWYYLHSTPIH---------V----RAPQFDLTGNGFT--W-------------------  446
BchE     378 PW---PFSDLF---------KELGDRVEVRDYSRYNFV--S-------------------  404
GenK     417 FWT-NPFYPIY-------------GSPDYQTCLSRGIV--D-------------------  441
Fom3     456 FF---AASPIV-------------GSELLRECIRQGFV--D-------------------  478
MaMmp10  232 PHTVSEFTEIVRSSAEKHPSIRITGTPLEDPLIGSPFAIRNVPEA--------LL-----  278

OxsB     561 RKEEVNLIMNDGKPDMNMGRYA--TSFVDK----TNGNLS--------------------  594
TsrM     450 ------LRM-CASPA----EYERQFMFD------EHGNFRL--VCYFS------PGLLPD  484
ThnK     419 EAETMDMVME--TSAMPRDEWVESFAFVQAVQFLHNGCYTRYLSVYLRQEHGIGYTAFYE  476
PhpK     447 ---------S---------HRT--MD---S----RQALEAA-------------DEIFD-  465
BchE     405 ---------PIIKPD-NMERE----D---V----LKGVLKS-------------YGRFY-  429
GenK     442 ---------PLTDPA-LFDQFN--FA---F----ANGVLAA-------------DELY--  467
Fom3     479 ---------PEESL------YR--MT---Y----KQGIINV-------------PG----  497
MaMmp10  279 -------KLP---------RVS-------KKATIITGQVAA-------------------  296

OxsB     184 SVNFGQK---------K-LAFEILDLIYSHIENGDLSSIITVGNVIPSFSPEQ-------  226
TsrM      67 TVRNTDTVYAFEQRPFVGEHREIITEIR-RLTD----APVVGGIGFSTMPFALV------  116
ThnK      71 SCYVWNF---------R-RQMKVAKILKERYPD----MLVVAGGPHVP-----------D  105
PhpK     120 TTTFYMM---------PAPVIEIIRFVRQHNAT----VPIIVGGPLI---DNHCREGRGE  163
BchE      71 TN-ITPS---------IFKAQDIMKIAKKVNKN----IRTIMGGIHSTFMYPQVLT----  112
GenK     116 SCMFTPY---------YESAYELARMAKRVLPN----AKVILGGQHGTVAFPHVL-----  157
Fom3     123 SSIFSNQ---------ADNVHHLLKLADLVTPE----AVTAIGGAHARYFPKACL-----  164
MaMmp10    0 ------------------------------------------------------------  0

OxsB     227 ----FFERYPS-LLICDKEGEYTLRDLIKMLKKEL---KLDEVNGISYVDESGEVKHNVA  278
TsrM     117 -------EYFGIEYGVKGPGEKILCELATAISEGR---DTAGIPGLIRNTERGAVRVPPA  166
ThnK     106 RVGDFFTQHPYVDVLAHGEGEVAFRELLSARLQDA--PVYTGVPGVTVNERNAPVAGPPA  163
PhpK     167 KLSRFFDRVGADFYVWESQGEEALAGLVGALVEGASPAAVPN----VFARADGEWKLGR-  218
BchE     113 -------EAPETDYVVRGEGEEVTVNLMKAIAAGNDKETRSEITGIAYIDENGKVFATAA  165
GenK     158 -------EVPEIDAVMLGEAEVTTVALLDAFATGR---PLTELLGVAFRCGEGLCDCPT-  206
Fom3     165 -------DDPNLDAVFLGEGEMTFLLWMEHLNGNV---SDDEVHGIAWRDRDGKVQIKPE  214
MaMmp10    0 ------------------------------------------------------------  0

OxsB     279 ETV---------------------NFKEEVPTPSLDILGEISKF----------------  301
TsrM     167 VLTVRHGK--------TQPAEPTGQF----EPRVWQV-DQLSVYRRRSGVPRKVDNLEYY  213
ThnK     164 KRL-----------------------PRRIETPSPYLNGVMDGAVKTC-----R----ER  191
PhpK     219 ------KR-------------PENNDLNR-ASVRWHRFGA-DVL----------------  241
BchE     166 HP--------------------VIEDLDTL-SPDWSLYDW-DKYIYTP------------  191
GenK     207 -PGTPHIRPR----------APFVADLDSLAPPAADQLDF-DRY----------------  238
Fom3     215 LPLISSMRPEGPETGKSSPMLSMAGELDHIPFPAWHHYNM-EKYFEIK-----AYQSPYT  268
MaMmp10    1 -------------------ME---VVVDVGGNPGVDCKGF-CKYCYFK---KVK---DIQ  31

OxsB     595 -------EACQMWIDSNFGVMYTI-------KSLHKVA------NPREKKKLYSY---ME  631
TsrM     485 --PARAADPEERWHGAV-------------------------------------------  499
ThnK     477 GLQNHFTDRPTSFIGSLYLRMRSLYQDYIDIPTLPLANLVAS--QPDLATHLAP----YG  530
PhpK     466 ---SV---HAASWMPVNGL------------DFWGVPY------LL---------GKGMS  492
BchE     430 ---ARKTFFSYPWIKDPYVRKYML-------GCLKAFA------KTTLTKRFYDIDRVKT  473
GenK     468 ----------TAWVGTLAM------------ALWPKYV------LEGAERR----EQGPV  495
Fom3     498 -----------LWDGEEIA------------ELAAKFN------RDFNARR----DRAYT  524
MaMmp10  297 ---SRLTEIFEALGGTVNV----I-PVKKDIGCLITIDDFKALDLSEVTET------VFI  342

OxsB     632 THREISHFLLKYLVYNLSPDKESQIILSDFLRMHSMEHILDNS--------KINVGDGSK  683
TsrM     500 ------ADLWALI----DPADHHRVMLPTVE---GMS-------------------E-HD  526
ThnK     531 RRRGWTIDNWGWLRVATEFDRFHD-ELVDYLRT-------------------LGLDPDQD  570
PhpK     493 REQVVE-FL---A-------------LAKPL---TTA----------------NVARSGP  516
BchE     474 KNRKIEIDL-GFD-------------QSRILTQEEAK----------------NLKERRP  503
GenK     496 SAAEAA---------------------ARLV-EHSMA----------------QLDPESP  517
Fom3     525 PQKQWN---------------------AN------------------------QY-----  534
MaMmp10  343 PGRAF----------------VHDMEIKEALRRDGVDRIVRRGPERLSVDGEMSIGMTRE  386

OxsB     684 ENILNVM-------------------------------------TNWQLIMEKLLR----  702
TsrM     527 NNYADN-P-FLTSLG-----GLGY---------------TGAFWSHWRGREEIMRKAREA  564
ThnK     571 ARLRDVLRFQRDV----MLR-PDYDA------QH---GKTAEYDHDWPGYFG--------  608
PhpK     517 EAAARAEARFRSFVGGLHLEPARYDAG----------------GQR--------------  546
BchE     504 EMIAD-------MSFGLK--EAGYQ--------------REHDEHNWDEFDESTI-----  535
GenK     518 EELPATVRAIRETADGLL--ALGHPLGCVCVMQHVADADKGAGADQFCRFAGDMIAAAIA  575
Fom3     535 ------------------------------------------------------------  534
MaMmp10  387 EVLELEVENFTELIGQIN--SLGLPLE---------------------------------  411

OxsB     302 --RGALTLETSRGCDYSRCTFCPRDHKLRS-WRPLSVEQTLKQLDDILRAGKHFNIKPHI  358
TsrM     214 RRGGLGSILTKNGCAY-RCSHCVEPDAKGTRYGQRELASV---VDEMESLAAQ-GILDQH  268
ThnK     192 GLRFYALWETNRGCPY-SCAFCDWGSATMSALRMFEEERV---QHEIEWFARN-DVE-DL  245
PhpK     242 --GRTVSTRTARSCAF-NCAFCDYPERAGA-LTLADLSTV---ERELEE-LAERGVKRVA  293
BchE     192 LNCRLAVPNFARGCPF-TCTFCSQWQFWR-RYRARSPKNF---VDEIEILVKKYKVGFFI  246
GenK     240 --GNAVTLITSRGCPF-SCSFCTVHATVGKQFRARDPQRV---VDEIEHYVNVHGVRRFL  292
Fom3     269 VGSRVGQLYTSRGCTA-HCTFCTTTHFWGQKLRRRSVQDV---VDEVLRLRDEYGIDEFH  324
MaMmp10   32 PLGCKYCLPFKKGCDY-----CTRSVKES-YSGFKSLQMVLEETANKLYFTSG-EVKKFT  84

OxsB     359 YMADEEFIGELPNGTEAQRIIDICEGLLKREEKIKFDFAARADSVYEPKRTKEWNVER--  416
TsrM     269 T-TDSEF------NLSIAHAKNLLREIVRRRHADPDNPLNR---------LRLWVYCQPS  312
ThnK     246 FICDANF-GIMPRDLEIAHALAEARTEAGSPKQIRVNFAKN---------SNDRVFDI--  293
PhpK     294 F-IDDTF------NVPMRRFKDLCRMMVRRDFGIEWFSYFR---------CGH-ARE---  333
BchE     247 L-ADEEP------TINKQKFVSLCQELIDRKLDVTWGINTR---------VTDIMRD---  287
GenK     293 V-EDDNF------TFDIERVHAICQEIVRRQLDVRLSLPNG---------MTVVKLT---  333
Fom3     325 I-QDDNI------TNDMDHARELFRAF--KEVGLPWATPQG---------TALWRMD---  363
MaMmp10   85 VSGGGDL-SCYPELKSLITFLSQFN------TPIHLGYTSG------------KGFSK--  123

OxsB     417 ---LKMWHYCALAGADRIFIGVESGSNQQLKRY-----GKGT-TSEQNIIALRLVSALGI  467
TsrM     313 PFDEEFADLLAAAGCRGVNVGSDHIRPELLSGWKVTEKGGTYYTFEDTERLVRLCRERGI  372
ThnK     294 ---SKAWHDADL--LMGTTLSMQSTDMDVLEAI-----DRKNIGLDNYRKLQQRYAVENI  343
PhpK     334 ---PGVYDLMYDSGCRGVLLGIESGDDQVLLNM-----DKRA-TTEHYRYGIEQLKARGI  384
BchE     288 ---EDLLPFYRKAGLVHVSLGTEAASQMNLNRF-----RKET-TIEENKYAIKLLQKNGI  338
GenK     334 ---EDLVESMVSAGFDDLFLGLETTDAARLRKM-----RKGFTSLDKVSAGVALFEKFGL  385
Fom3     364 ---EELLDLMAESGAYQVTFAIESGVQRVLKEL-----IKKPLNLERTSHLIKYARSLGM  415
MaMmp10  124 ---PDDALFYIDNGVTEVSFTVFATDPALRAEY-----MKDP----EPEASIQVLRDFCT  171

OxsB     703 -------------DVEADLNKG---IITDS-EDHR---LHNTLKR-WFSDMGN----WSL  737
TsrM     565 AAQS---------------------SHLPTPV----------------------------  575
ThnK     609 ---GGPLRTQRVRVEYTDQQFGANGRFRPAPGDLKAFALAAIGPSYPVSRIGHYRH--RI  663
PhpK     547 ------------------------------------------------------------  546
BchE     536 ------------RDRTSSTVRNC-------------------------------------  546
GenK     576 LYSGQPQVSAQVGAQTAGDAEGCSFMVRPT-GDER---IGRIQRR-FVELLDENRRDAEL  630
Fom3     534 ------------------------------------------------------------  534
MaMmp10  411 ------------------------------------------------------------  411
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Figure S4. Truncated sequence alignment of Class B radical SAM methyltransferases. Highlighted in grey is the predicted or known cobalamin binding domain from Uniprot (1). Highlighted in blue is the SAM binding domain of OxsB (2). The conserved cysteines of the radical SAM domain are highlighted in yellow. The residues from OxsB shown to interact with cobalamin are shown with white lettering and highlighted in navy. The organisms are as follows for the alignment from top to bottom with the accession number and percent identity to OxsB in parenthesis: *Bacillus megaterium* (O24770), *Kitasatospora setae* (E4N8S5, 16.5%), *Streptomyces pratensis* (E8W2A9, 17.0%), *Kitasatospora phosalacinea* (A0A0M3N271, 16.4%), *Chlorobaculum tepidum* (H2VFK1, 23.0%), *Micromonospora echinospora* (A0A0K0K526, 18.1%), *Streptomyces wedmorensis* (Q56184, 18.0%), and *Methanosarcina acetivorans* (Q8THG6, 15.1%).


PDZ domain
CXXXCXXC
Aldolase-Type TIM barrel
DUF512
Q8THG6
Q6LX00
A0A0K2HHB4
A0A0A1ZFH5
A0A068MV33
A0A367RII2
Q182W7
A0A072X0Q4
A0A0H2YRV6
A0A0B5QA96
A0A2U3DCR3
A0A1H0HVY8
A0A173UKJ0
A0A0S2W3K9
A0A1Y4LMK6
A0A0S6UD50
Q8TVA4
A0A016QUC2
A0A2Z2M7L8
A0A0S1XAH9
I6U6R8
O58868

Figure S5. Truncated sequence alignment of *Ma*Mmp10 and *Mm*Mmp10 with representative DUF512 family members. DUF512 sequences were chosen at random from more than the 3,000 from InterPro (3). Red highlighting represents 100% amino acid conservation between the twenty-two sequences in the alignment. Grey, dark cyan, light blue, and olive green highlighting represents amino acid conservation greater than 85% but less than 100% where grey represents the hydrophobic amino acids A, I, L, V, M, P, and G; dark cyan represents the aromatic amino acids F, W, and Y; light blue represents the charged amino acids D, E, H, R, and K; and olive green for S and T. The residues in red text represent conservation greater than 25% and less than 85% between *Ma*Mmp10 and the DUF512 proteins. Q, N, and C did not show conservation greater than 25% except for the cysteines in the radical SAM motif, denoted by CXXXCXXC above the aligned sequences. The PDZ, Aldolase-type TIM barrel, and DUF512 domains are generalized for all of the sequences and were determined from InterPro (3). The accession numbers in the sequence alignment correspond to the following organisms with the percent identity of each sequence to *Ma*Mmp10 in parenthesis: Q8THG6, *Ma*Mmp10; Q6LX00, *Methanococcus maripaludis* S2 (45.9%); A0A0K2HHB4, *Bacteroides dorei* (13.4%); A0A0A1ZFH5, *Clostridium marinus* str. MIT 9116 (17.9%); A0A068MV33, *Synechococcus* sp. (PCC 6714) (18.9%); A0A367RII2, *Nostoc punctiforme* NIES-2108 (20.3%); Q182W7, *Peptoclostridium difficile* str. 630 (18.8%); A0A072X0Q4, *Clostridium botulinum* C/D str. It1 (18.1%); A0A0H2YRV6, *Clostridium perfringens* (ATCC 131124) (19.8%); A0A0B5QA96, *Clostridium beijerinckii* (16.8%); A0A2U3DCR3, *Acidibacillus sulfuroxidans* (17.2%); A0A1H0HVY8, *Eubacterium limosum* (20.0%); A0A173UKJ0, *Faecalibacterium prausnitizii* (19.4%); A0A0S2W3K9, *Intestinimonas butyriciproducens* (20.8%); A0A1Y4LMK6, *Butyricicoccus pullicaecorum* (20.8%); A0A0S6UD50, *Moorella thermoacetica* Y72 (22.8%); Q8TVA4, *Methanopyrus kandleri* DSM 6324 (21.5%); A0A016QUC2, *Deinococcus puniceus* (17.9%); A0A2Z2M7L8, *Thermococcus gorgonarius* (20.0%); A0A0S1XAH9, *Thermococcus barophilus* (20.6%); I6U6R8, *Pyrococcus furiosus* COM1 (20.0%); O58868, *Pyrococcus horikoshii* ATCC 700860 (22.1%).

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ATGGAAGTTGTTGTTGACGTTGGTGGTAATCCGGGTGTTGATTGTAAAGGCTTTTGCAAATACTGCTATT
TCAAAAAAGTGAAAGACATTCAGCCGCTGGGTTGTAAATATTGTCTGCCGTTTAAAAAGGGCTGCGATTA
TTGTACCCGTAGCGTTAAAGAAAGCTATAGCGGTTTTAAAAGCCTGCAGATGGTTCTGGAAGAAACCGCA
AATAAACTGTATTTTACCAGCGGTGAGGTGAAAAAATTCACCGTTAGCGGTGGTGGTGATCTGAGCTGTT
ATCCGGAACTGAAAAGCCTGATTACCTTTCTGAGCCAGTTTAATACCCCGATTCATCTGGGTTATACCAG
CGGCAAAGGTTTTAGCAAACCGGATGATGCACTGTTCTATATTGATAATGGTGTTACCGAAGTGAGCTTT
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ACCGCTGGAAGATCCGCTGATTGGTAGCCCGTTTGCAATTCGTAATGTTCCGGAAGCACTGCTGAAACTG
CCTCGTGTTAGCAAAAAAGCAACCATTATTACAGGTCAGGTTGCAGCAAGCCGTCTGACCGAAATTTTTG
AAGCATTAGGTGGCACCGTTAATGTGATCCCGGTTAAAAAAGATATTGGTTGCCTGATCACCATCGATGA
TTTTAAAGCACTGGATCTGAGCGAAGTGACAGAAACCGTTTTTATTCCTGGTCGTGCATTTGTGCATGAT
ATGGAAATCAAAGAAGCCCTGCGACGCGACGGTGTTGATCGTATTGTTCGTCGTGGTCCGGAACGTCTGA
GCGTGGATGGTGAAATGAGCATTGGTATGACCCGTGAAGAAGTTCTGGAACTGGAAGTTGAAAACTTTAC
CGAACTGATTGGCCAGATTAATAGCCTGGGTCTGCCGCTGGAA
```

Figure S6. Gene sequence of codon-optimized *Ma*Mmp10.

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