Supplemental Information

Title: Structural basis for non-radical catalysis by TsrM, a radical SAM methylase

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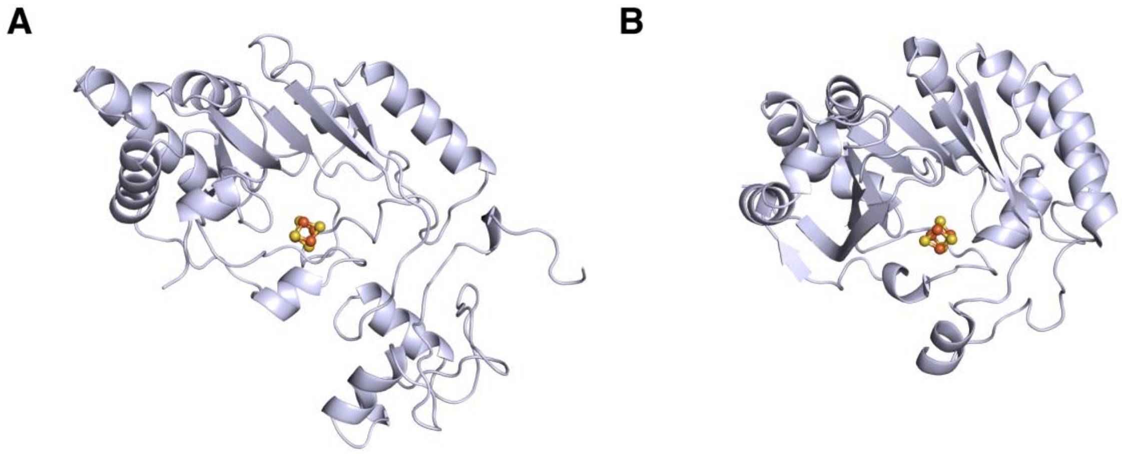
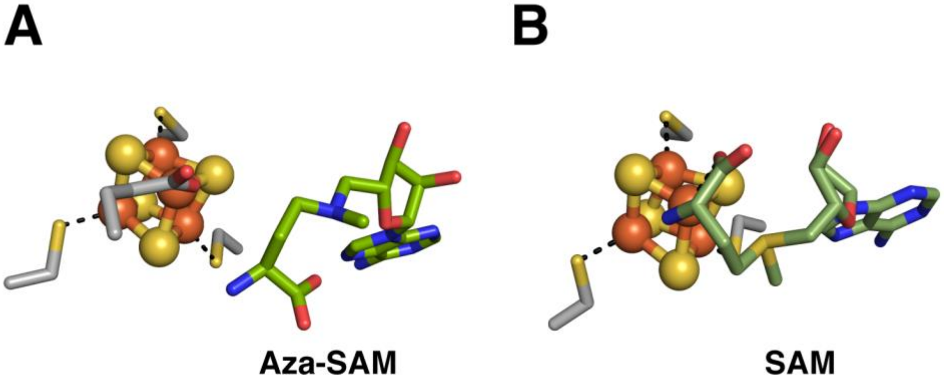
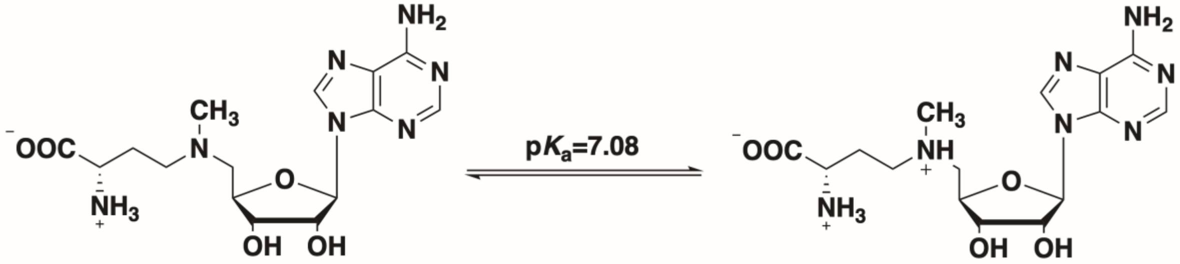


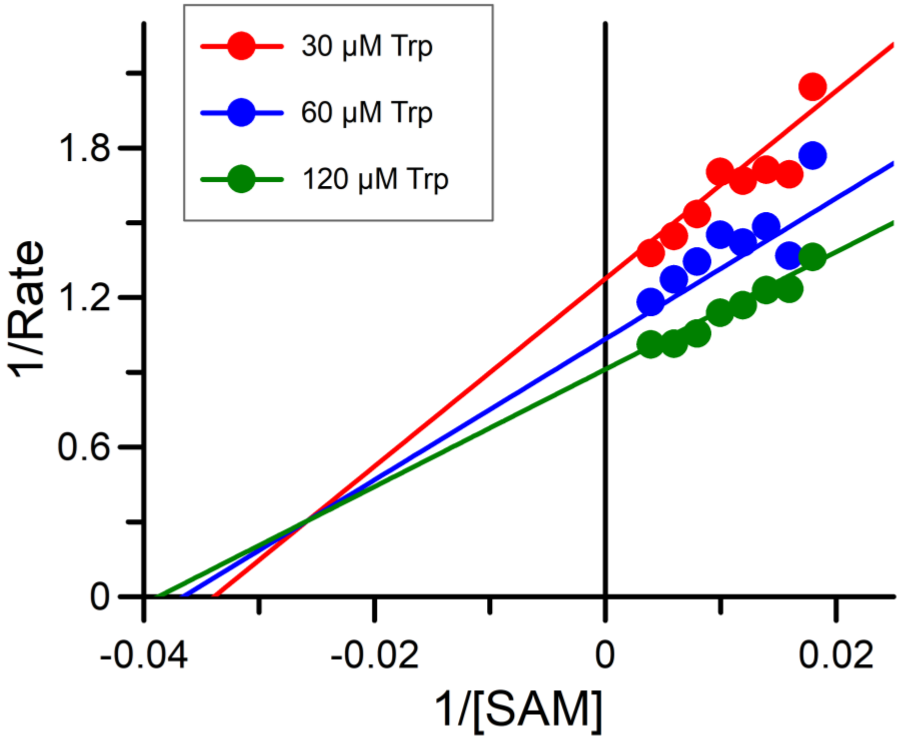
Supplementary Figure 1. Comparison of RS core folds of (**A**) *Ks*TsrM and (**B**) *E. coli* pyruvate formate-lyase activating enzyme (PDB: 3CF8).



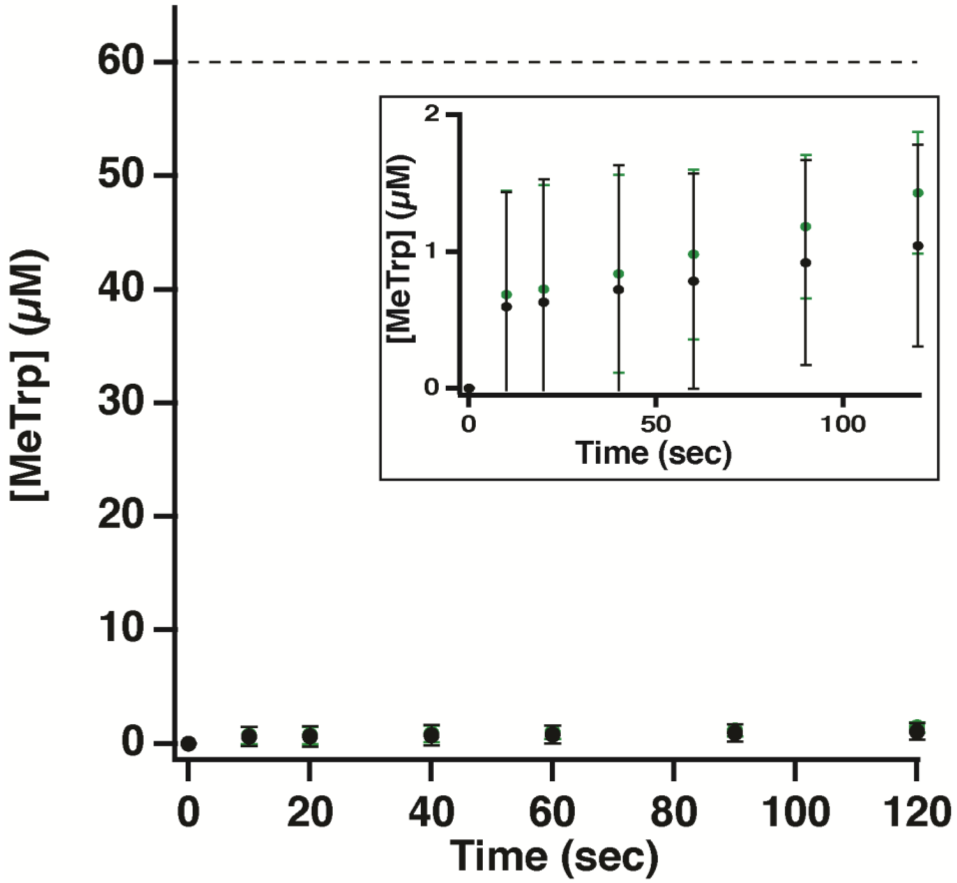
Supplementary Figure 2. Comparison of (**A**) aza-SAM binding in *Ks*TsrM and (**B**) SAM binding in RlmN (pdb:3RFA). In this figure, the orientation of the cluster is maintained to show better the differences in the SAM and aza-SAM conformations.



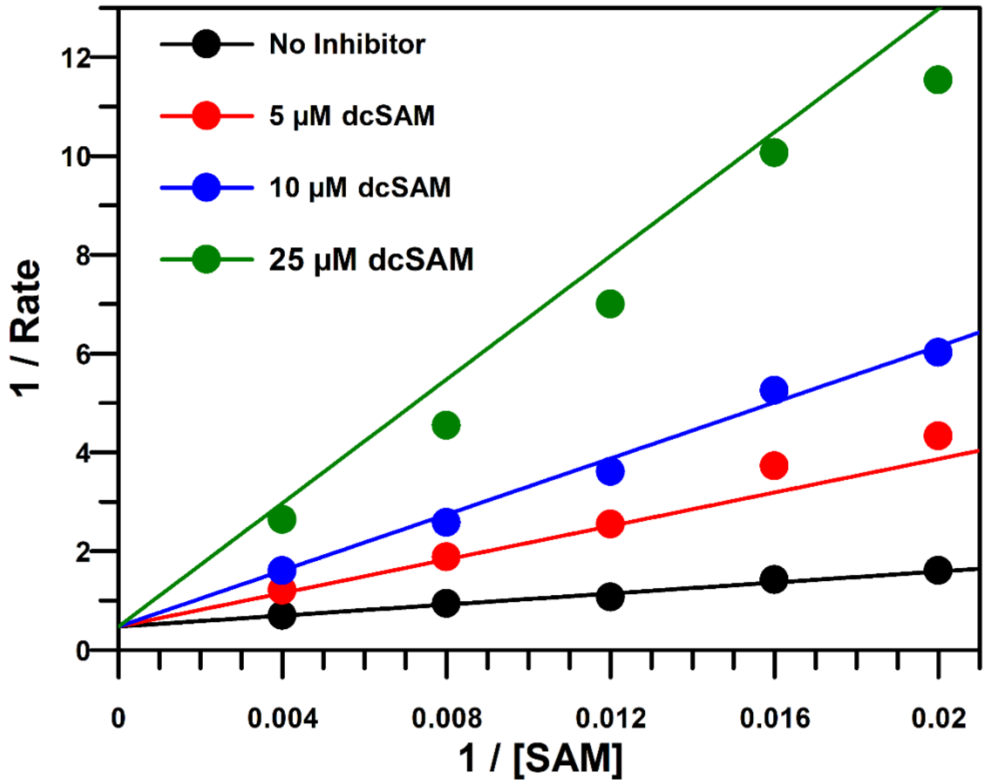
Supplementary Figure 3. Structure of aza-SAM (S-5'-azamethionine-5-deoxyadenosine).



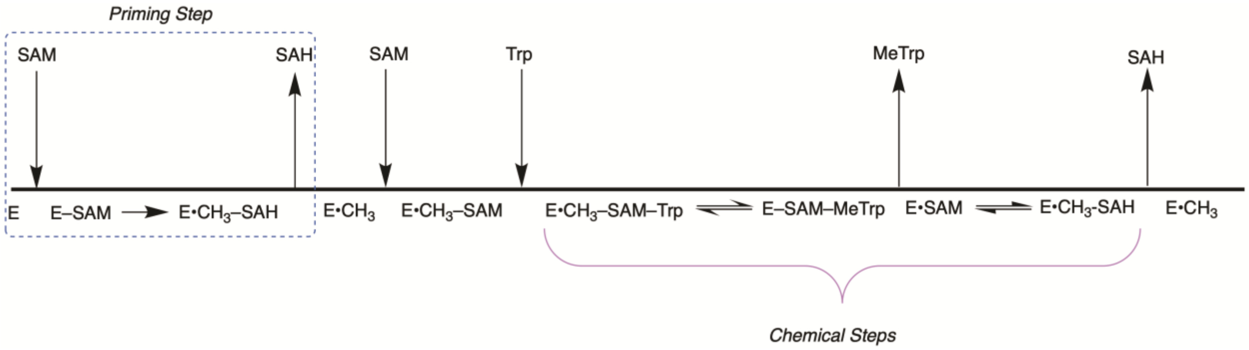
Supplementary Figure 4. Double reciprocal plots of the initial rates of MeTrp formation at varying concentrations of SAM (55 – 250 µM) and fixed concentrations of Trp (30, 60, and 90 µM). The data were fitted globally to Equation 1 using GraFit (Erithacus Software).



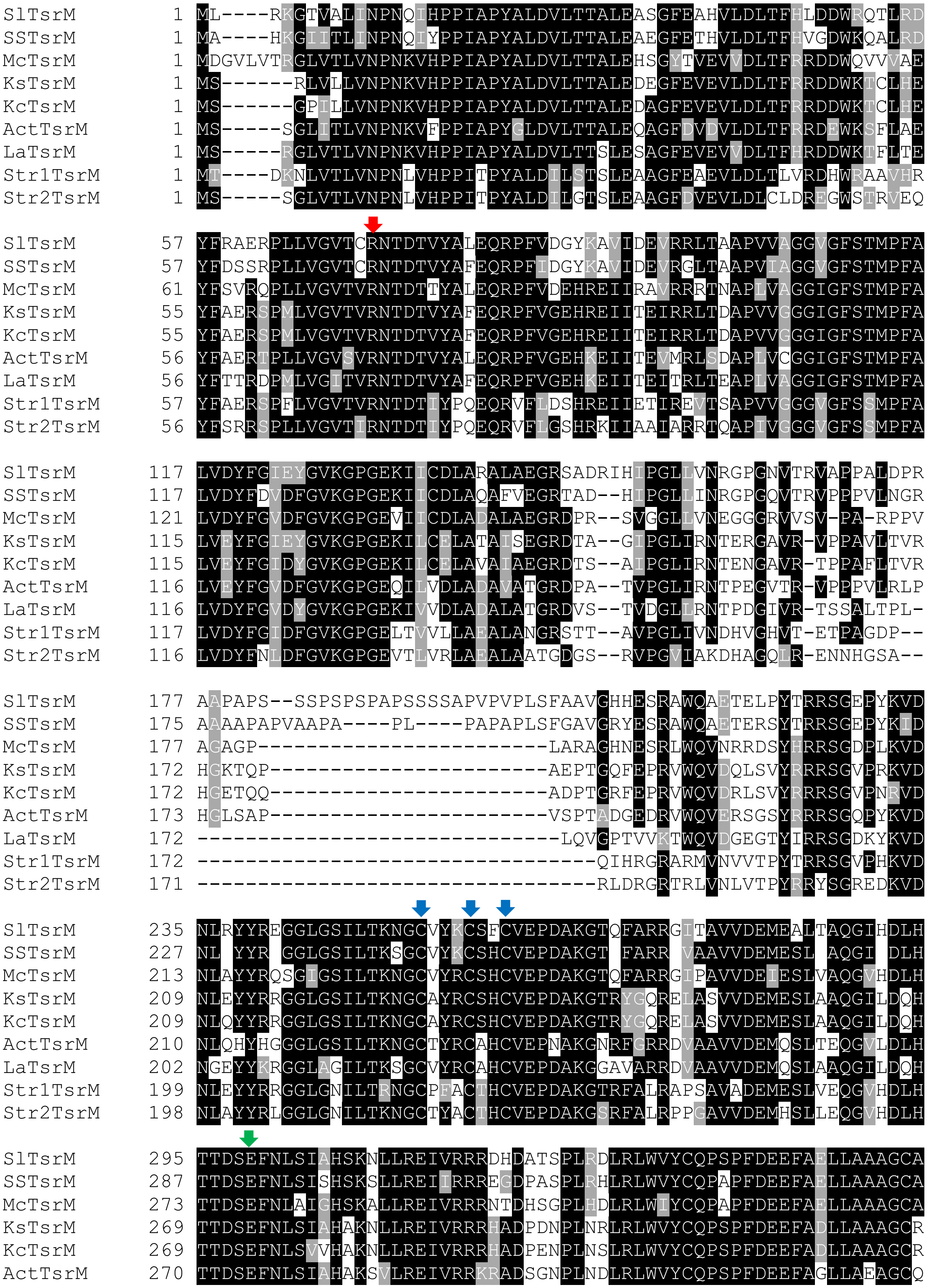
Supplementary Figure 5. Time-dependent formation of MeTrp under single-turnover conditions. Reactions contained premethylated TsrM (60 μM) and no SAM (black dots) or dcSAM (1 mM) (green dots). The inset represents a reduced Y-axis scale to allow better visualization of the activity. Error bars represent the standard deviation of triplicate determinations, with the central point representing the average.



Supplementary Figure 6. Double-reciprocal plots of the initial rates of MeTrp formation at varying concentrations of SAM and dcSAM. The data were fitted globally to Equation 2 using GraFit (Erithacus Software).



Supplementary Figure 7. Kinetic hypothesis for the TsrM reaction. In the priming step, SAM and a reducing agent are used to convert OHCbl or cob(II)alamin to MeCbl (E–CH_3). Upon release of SAH, a second SAM molecule binds to the E–CH_3 state of TsrM, which is followed by the binding of Trp. Methyltransfer from MeCbl to Trp takes place with assistance from SAM (chemical steps), affording cob(I)alamin. Upon release of MeTrp, SAM methylates cob(I)alamin to regenerate MeCbl, and SAH is released.



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SSTsrM       1 MA----HKGIITLINPNQIYPPIAPYALDVLTTALEAEGFETHVLDLTFHVGDWKQALRD
McTsrM       1 MDGVLVTRGLVTLVNPNKVHPPIAPYALDVLTTALEHSGYTVEVVDLTFRRDDWQVVVAE
KsTsrM       1 MS------RLVLLVNPNKVHPPIAPYALDVLTTALEDEGFEVEVLDLTFRRDDWKTCLHE
KcTsrM       1 MS------GPILLVNPNKVHPPIAPYALDVLTTALEDAGFEVEVLDLTFRRDDWKTCLHE
ActTsrM      1 MS-----SGLITLVNPNKVFPPIAPYGLDVLTTALEQAGFDVDVLDLTFRRDEWKSFLAE
LaTsrM       1 MS-----RGLVTLVNPNKVHPPIAPYALDVLTTSLESAGFEVEVVDLTFHRDDWKTFLTE
Str1TsrM     1 MT----DKNLVTLVNPNLVHPPITPYALDILSTSLEAAGFEAEVLDLTLVRDHWRAAVHR
Str2TsrM     1 MS-----SGLVTLVNPNLVHPPITPYALDILGTSLEAAGFDVEVLDLCLDREGWSTRVEQ

SlTsrM      57 YFRAERPLLVGVTCRNTDTVYALEQRPFVDGYKAVIDEVRRLTAAPVVAGGVGFSTMPFA
SSTsrM      57 YFDSSRPLLVGVTCRNTDTVYAFEQRPFIDGYKAVIDEVRGLTAAPVIAGGVGFSTMPFA
McTsrM      61 YFSVRQPLLVGVTVRNTDTTYALEQRPFVDEHREIIRAVRRRTNAPLVAGGIGFSTMPFA
KsTsrM      55 YFAERSPMLVGVTVRNTDTVYAFEQRPFVGEHREIITEIRRLTDAPVVGGGIGFSTMPFA
KcTsrM      55 YFAERSPMLVGVTVRNTDTVYAFEQRPFVGEHREIITEIRRLTDAPVVGGGIGFSTMPFA
ActTsrM     56 YFAERTPLLVGVSVRNTDTVYALEQRPFVGEHKEIITEVMRLSDAPLVCGGIGFSTMPFA
LaTsrM      56 YFTTRDPMLVGITVRNTDTVYAFEQRPFVGEHKEIITEITRLTEAPLVAGGIGFSTMPFA
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Str2TsrM    56 YFSRRSPLLVGVTIRNTDTIYPQEQRVFLGSHRKIIAAIARRTQAPIVGGGVGFSSMPFA

SlTsrM     117 LVDYFGIEYGVKGPGEKIICDLARALAEGRSADRIHIPGLLVNRGPGNVTRVAPPALDPR
SSTsrM     117 LVDYFDVDFGVKGPGEKIICDLAQAFVEGRTAD--HIPGLLINRGPGQVTRVPPPVLNGR
McTsrM     121 LVDYFGVDFGVKGPGEVIICDLADALAEGRDPR--SVGGLLVNEGGGRVVSV-PA-RPPV
KsTsrM     115 LVEYFGIEYGVKGPGEKILCELATAISEGRDTA--GIPGLIRNTERGAVR-VPPAVLTVR
KcTsrM     115 LVEYFGIDYGVKGPGEKILCELAVAIAEGRDTS--AIPGLIRNTENGAVR-TPPAFLTVR
ActTsrM    116 LVEYFGVDFGVKGPGEQILVDLADAVATGRDPA--TVPGLIRNTPEGVTR-VPPPVLRLP
LaTsrM     116 LVDYFGVDYGVKGPGEKIVVDLADALATGRDVS--TVDGLLRNTPDGIVR-TSSALTPL-
Str1TsrM   117 LVDYFGIDFGVKGPGELTVVLLAEALANGRSTT--AVPGLIVNDHVGHVT-ETPAGDP--
Str2TsrM   116 LVDYFNLDFGVKGPGEVTLVRLAEALAATGDGS--RVPGVIAKDHAGQLR-ENNHGSA--

SlTsrM     177 AAPAPS--SSPSPSPAPSSSSAPVPVPLSFAAVGHHESRAWQAETELPYTRRSGEPYKVD
SSTsrM     175 AAAAPAPVAAPA----PL----PAPAPLSFGAVGRYESRAWQAETERSYTRRSGEPYKID
McTsrM     177 AGAGP------------------------LARAGHNESRLWQVNRRDSYHRRSGDPLKVD
KsTsrM     172 HGKTQP-----------------------AEPTGQFEPRVWQVDQLSVYRRRSGVPRKVD
KcTsrM     172 HGETQQ-----------------------ADPTGRFEPRVWQVDRLSVYRRRSGVPNRVD
ActTsrM    173 HGLSAP-----------------------VSPTADGEDRVWQVERSGSYRRRSGQPYKVD
LaTsrM     172 ------------------------------LQVGPTVVKTWQVDGEGTYIRRSGDKYKVD
Str1TsrM   172 ---------------------------------QIHRGRARMVNVVTPYTRRSGVPHKVD
Str2TsrM   171 ---------------------------------RLDRGRTRLVNLVTPYRRYSGREDKVD

SlTsrM     235 NLRYYREGGLGSILTKNGCVYKCSFCVEPDAKGTQFARRGITAVVDEMEALTAQGIHDLH
SSTsrM     227 NL YYR GGLGSILTKSGCVYKCSHCVEPDAKGT FARR VAAVVDEMESL AQGI DLH
McTsrM     213 NLAYYRQSGIGSILTKNGCVYRCSHCVEPDAKGTQFARRGIPAVVDEIESLVAQGVHDLH
KsTsrM     209 NLEYYRRGGLGSILTKNGCAYRCSHCVEPDAKGTRYGQRELASVVDEMESLAAQGILDQH
KcTsrM     209 NLQYYRRGGLGSILTKNGCAYRCSHCVEPDAKGTRYGQRELASVVDEMESLAAQGILDQH
ActTsrM    210 NLQHYHGGGLGSILTKNGCTYRCAHCVEPNAKGNRFGRRDVAAVVDEMQSLTEQGVLDLH
LaTsrM     202 NGEYYKRGGLAGILTKSGCVYRCAHCVEPDAKGGAVARRDVAAVVDEMQSLAAQGILDQH
Str1TsrM   199 NLEYYRRGGLGNILTRNGCPFACTHCVEPDAKGTRFALRAPSAVADEMESLVEQGVHDLH
Str2TsrM   198 NLAYYRLGGLGNILTKNGCTYACTHCVEPDAKGSRFALRPPGAVVDEMHSLLEQGVHDLH

SlTsrM     295 TTDSEFNLSIAHSKNLLREIVRRRDHDATSPLRDLRLWVYCQPSPFDEEFAELLAAAGCA
SSTsrM     287 TTDSEFNLSISHSKSLLREIIRRREGDPASPLRHLRLWVYCQPAPFDEEFAELLAAAGCA
McTsrM     273 TTDSEFNLAIGHSKALLREIVRRRNTDHSGPLHDLRLWIYCQPAPFDEEFAELLAAAGCA
KsTsrM     269 TTDSEFNLSIAHAKNLLREIVRRRHADPDNPLNRLRLWVYCQPSPFDEEFADLLAAAGCR
KcTsrM     269 TTDSEFNLSVVHAKNLLREIVRRRHADPENPLNSLRLWVYCQPSPFDEEFADLLAAAGCR
ActTsrM    270 TTDSEFNLSIAHAKSVLREIVRRKRADSGNPLNDLRLWVYCQPSPFDEEFAGLLAEAGCQ
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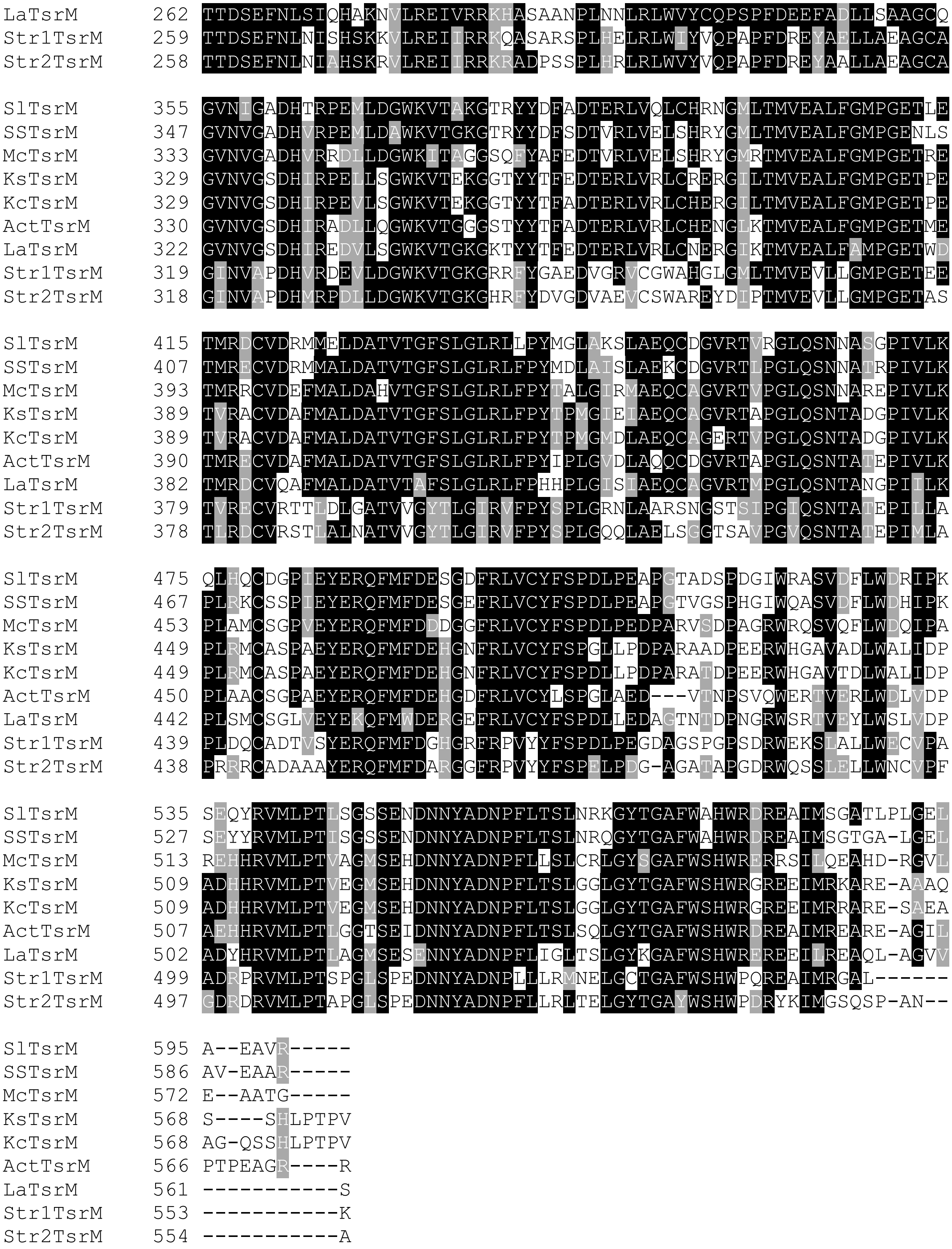

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Str2TsrM   258 TTDSEFNLNIAHSKRVLREIIRRKRADPSSPLHRLRLWVYVQPAPFDREYAALLAEAGCA

SlTsrM     355 GVNIGADHTRPEMLDGWKVTAKGTRYYDFADTERLVQLCHRNGMLTMVEALFGMPGETLE
SSTsrM     347 GVNVGADHVRPEMLDAWKVTGKGTRYYDFSDTVRLVELSHRYGMLTMVEALFGMPGENLS
McTsrM     333 GVNVGADHVRRDLLDGWKITAGGSQFYAFEDTVRLVELSHRYGMRTMVEALFGMPGETRE
KsTsrM     329 GVNVGSDHIRPELLSGWKVTEKGGTYYTFEDTERLVRLCRERGILTMVEALFGMPGETPE
KcTsrM     329 GVNVGSDHIRPEVLSGWKVTEKGGTYYTFADTERLVRLCHERGILTMVEALFGMPGETPE
ActTsrM    330 GVNVGSDHIRADLLQGWKVTGGGSTYYTFADTERLVRLCHENGLKTMVEALFGMPGETME
LaTsrM     322 GVNVGSDHIREDVLSGWKVTGKGKTYYTFEDTERLVRLCNERGIKTMVEALFAMPGETWD
Str1TsrM   319 GINVAPDHVRDEVLDGWKVTGKGRRFYGAEDVGRVCGWAHGLGMLTMVEVLLGMPGETEE
Str2TsrM   318 GINVAPDHMRPDLLDGWKVTGKGHRFYDVGDVAEVCSWAREYDIPTMVEVLLGMPGETAS

SlTsrM     415 TMRDCVDRMMELDATVTGFSLGLRLLPYMGLAKSLAEQCDGVRTVRGLQSNNASGPIVLK
SSTsrM     407 TMRECVDRMMALDATVTGFSLGLRLFPYMDLAISLAEKCDGVRTLPGLQSNNATRPIVLK
McTsrM     393 TMRRCVDEFMALDAHVTGFSLGLRLFPYTALGIRMAEQCAGVRTVPGLQSNNAREPIVLK
KsTsrM     389 TVRACVDAFMALDATVTGFSLGLRLFPYTPMGIEIAEQCAGVRTAPGLQSNTADGPIVLK
KcTsrM     389 TVRACVDAFMALDATVTGFSLGLRLFPYTPMGMDLAEQCAGERTVPGLQSNTADGPIVLK
ActTsrM    390 TMRECVDAFMALDATVTGFSLGLRLFPYIPLGVDLAQQCDGVRTAPGLQSNTATEPIVLK
LaTsrM     382 TMRDCVQAFMALDATVTAFSLGLRLFPHHPLGISIAEQCAGVRTMPGLQSNTANGPIILK
Str1TsrM   379 TVRECVRTTLDLGATVVGYTLGIRVFPYSPLGRNLAARSNGSTSIPGIQSNTATEPILLA
Str2TsrM   378 TLRDCVRSTLALNATVVGYTLGIRVFPYSPLGQQLAELSGGTSAVPGVQSNTATEPIMLA

SlTsrM     475 QLHQCDGPIEYERQFMFDESGDFRLVCYFSPDLPEAPGTADSPDGIWRASVDFLWDRIPK
SSTsrM     467 PLRKCSSPIEYERQFMFDESGEFRLVCYFSPDLPEAPGTVGSPHGIWQASVDFLWDHIPK
McTsrM     453 PLAMCSGPVEYERQFMFDDDGGFRLVCYFSPDLPEDPARVSDPAGRWRQSVQFLWDQIPA
KsTsrM     449 PLRMCASPAEYERQFMFDEHGNFRLVCYFSPGLLPDPARAADPEERWHGAVADLWALIDP
KcTsrM     449 PLRMCASPAEYERQFMFDEHGNFRLVCYFSPDLLPDPARATDPEERWHGAVTDLWALIDP
ActTsrM    450 PLAACSGPAEYERQFMFDEHGDFRLVCYLSPGLAED---VTNPSVQWERTVERLWDLVDP
LaTsrM     442 PLSMCSGLVEYEKQFMWDERGEFRLVCYFSPDLLEDAGTNTDPNGRWSRTVEYLWSLVDP
Str1TsrM   439 PLDQCADTVSYERQFMFDGHGRFRPVYYFSPDLPEGDAGSPGPSDRWEKSLALLWECVPA
Str2TsrM   438 PRRRCADAAAYERQFMFDARGGFRPVYYFSPELPDG-AGATAPGDRWQSSLELLWNCVPF

SlTsrM     535 SEQYRVMLPTLSGSSENDNNYADNPFLTSLNRKGYTGAFWAHWRDREAIMSGATLPLGEL
SSTsrM     527 SEYYRVMLPTISGSSENDNNYADNPFLTSLNRQGYTGAFWAHWRDREAIMSGTGA-LGEL
McTsrM     513 REHHRVMLPTVAGMSEHDNNYADNPFLLSLCRLGYSGAFWSHWRERRSILQEAHD-RGVL
KsTsrM     509 ADHHRVMLPTVEGMSEHDNNYADNPFLTSLGGLGYTGAFWSHWRGREEIMRKARE-AAAQ
KcTsrM     509 ADHHRVMLPTVEGMSEHDNNYADNPFLTSLGGLGYTGAFWSHWRGREEIMRRARE-SAEA
ActTsrM    507 AEHHRVMLPTLGGTSEIDNNYADNPFLTSLSQLGYTGAFWSHWRDREAIMREARE-AGIL
LaTsrM     502 ADYHRVMLPTLAGMSESENNYADNPFLIGLTSLGYKGAFWSHWREREEILREAQL-AGVV
Str1TsrM   499 ADRPRVMLPTSPGLSPEDNNYADNPLLLRMNELGCTGAFWSHWPQREAIMRGAL------
Str2TsrM   497 GDRDRVMLPTAPGLSPEDNNYADNPFLLRLTELGYTGAYWSHWPDRYKIMGSQSP-AN--

SlTsrM     595 A--EAVR-----
SSTsrM     586 AV-EAAR-----
McTsrM     572 E--AATG-----
KsTsrM     568 S----SHLPTPV
KcTsrM     568 AG-QSSHLPTPV
ActTsrM    566 PTPEAGR----R
LaTsrM     561 -----------S
Str1TsrM   553 -----------K
Str2TsrM   554 -----------A
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Supplementary Figure 8. Alignment of nine TsrM homologs with associated UniProt accession codes. Black indicates strictly conserved, light grey is partially conserved. The homologs are from the following species, with indicated percent identity relative to TsrM from *Streptomyces laurentti*: *Streptomyces laurentti* (SlTsrM, C0JRZ9, 100%), *Streptomyces sioyaensis* (SSTsrM (SioT), C0JRX4, 81.3%), *Micromonospora carbonacea* (McTsrM, A0A1C4YSC7, 66.8%), *Kitasatospora setae* (KsTsrM, E4N8S5, 64.9%), *Kitasatospora cheerisanensis* (KcTsrM, A0A066YXN6, 64.7%), *Actinoplanes* sp. (ActTsrM, strain ATCC 31044) (G8SKH5, 63.7%), *Lechevalieria aerocolonigenes* (LaTsrM, A0A0F0H1M0, 61.0%), *Streptomyces* sp. AA4 (Str1TsrM, D9V2D1, 55.0%), and *Streptomyces* AA4 (Str2TsrM, D9VIE6, 55.0%). The conserved Arg residue is indicated by a red arrow above. The CXXXCXXC motif is indicated by the three blue arrows above. The conserved Glu is shown by a green arrow above.

Supplementary Data Table 1. Data collection and refinement statistics for *Ks*TsrM with and without substrate bound.

	Anomalous Data**	Native *Ks*TsrM	HR Native *Ks*TsrM	*Ks*TsrM + azaSAM + Trp
Data collection				
Space group	*P* 2_1	*P* 2_1	*P* 2_1	*P* 2_1
Cell dimensions				
a, *b*, *c* (Å)	49.33, 104.25, 106.30	49.20, 104.02, 106.05	49.37, 103.76, 105.97	50.36, 103.08, 105.76
α, β, γ (°)	95.12	95.13	95.08	94.77
Resolution* (Å)	30.0-2.50 (2.54-2.50)	50.0-2.00 (2.07-2.00)	50.0-1.67 (1.73-1.67)	50.0-2.19 (2.27-2.19)
R_{sym} or $R_{\mathrm{merge}*}$	0.08 (0.23)	0.07 (0.14)	0.12 (0.82)	0.17 (0.70)
$I / \sigma I$*	11.53 (3.92)	9.13 (3.95)	11.15 (2.06)	6.47 (1.78)
Completeness (%)*	92.7 (54.0)	95.0 (77.0)	99.9 (99.6)	97.1 (96.8)
Redundancy*	2.9 (1.8)	2.3 (1.5)	6.1 (5.7)	3.2 (3.2)
Refinement				
Resolution (Å)		1.99	1.67	2.19
No. reflections		67,947	122,903	53,942
R_{work} / R_{free}		0.17/0.21	0.19/0.22	0.21/0.25
No. atoms		9,709	9,802	9,174
Protein		8,679	8,814	8,515
Ligand/ion		201	270	282
Water		830	718	377
B-factors (Å^2)				
Protein		24.6	19.8	23.4
Ligand/ion		18.5	17.8	18.1
Water		29.3	24.8	23.2
R.m.s. deviations				
Bond lengths (Å)		0.007	0.004	0.004
Bond angles (°)		1.37	0.73	0.67
Number of TLS groups		-	2	-
PDB accession code			6WTE	6WTF

*Values in parentheses are for highest-resolution shell. **Friedel pairs scaled separately. ***all structures result from one single crystal