Supporting Information

Adenylation and *S*-methylation of cysteine by the bifunctional enzyme TioN in thiocoraline biosynthesis

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Bacterial strains, materials, and instrumentation. R5A production medium (adjusted to pH 6.85 using KOH) contains per L: sucrose (103 g), K_2SO_4 (0.25 g), $MgCl_2 \cdot H_2O$ (10.12 g), glucose (10 g), casamino acids (0.1g), yeast extract (5 g), MOPS (21 g), and trace elements solution (2 mL) (as for R5 medium).[1] HPLC-ESI-MS analyses monitoring thiocoraline production in *S. albus*-pFL1049, *S. albus*-pFL1049-Δ*tioN*, and *S. albus*-pFL1049-Δ*tioT* were performed using a Shimadzu HPLC-MS system equipped with two LC-10AD pumps and a UV-Vis SPD-M10AD photo-diode array detector using a C18 ODS1 Teknochroma column (0.46 × 25 cm, 5 μm particle size) (for Δ*tioN*) and an UHPLC Agilent 1260 Infinity 600 equipped with a quaternary pump and a 1260 Infinity photo-diode array detector using a C18 Extrasil Teknochroma column (0.46 × 25 cm, 5 μm particle size) (for Δ*tioT*). Restriction endonucleases, T4 DNA ligase, as well as Pfx and Phusion DNA polymerase were purchased either from Invitrogen or New England BioLabs. DNA primers for PCR were bought from Integrated DNA Technologies. The pET28a and pACYCDuet-1 overexpression vectors were purchased from Novagen. Chemically competent *E. coli* TOP10 and DH10B cells were purchased from Invitrogen. The BL21 (DE3)*ybdZ*::*aac(3)IV* bacterial strain used for co-expression of TioN and TioT was provided by Prof. Michael G. Thomas (University of Wisconsin-Madison, WI).[2] DNA sequencing for the *tioN* and *tioT* knockouts as well as for their expression constructs was performed at the SCTs at the Universidad de Oviedo and at the University of Michigan DNA Sequencing Core, respectively. All chemicals and buffer components were purchased from Sigma-Aldrich or VWR and used without any further purification. [^{32}P]PP_i, [^{35}S]L-Cys, and [methyl-^{3}H]SAM (*S*-adenosyl-L-methionine) were purchased from Perkin Elmer. The TioS(T_4) protein was overexpressed and purified as previously described.[3]

Generation of a *tioN* knockout in *S. albus*-pFL1049. The preparation of the *tioN* mutant strain (Δ*tioN*) is depicted in Fig. S1. A 1,553-bp DNA fragment in the *tioN* gene sequence was PCR-amplified using oligonucleotides GVR-N-up (5'-AAACGTACCTTCACCTGGTCGTC-3') and GVR-N-rp (5'-AAACGACCTCGTGGTGAACTTCT-3'). The PCR reaction mixture contained genomic DNA from *S. albus*-pFL1049 (1 μL), the oligonucleotides (20 pmol/μL stock solution) (1.5 μL of each primer), dNTPs (2 mM each) (7.5 μL), $MgSO_4$ (50 mM) (1 μL), Pfx reaction buffer (10x) (5 μL), Pfx enhancer solution (10x) (10 μL), distilled H_2O (22.5 μL), and Pfx DNA polymerase (0.5 μL). *S. albus*-pFL1049 is a thiocoraline heterologous producer strain containing the whole 52,908 bp-long biosynthetic gene cluster for thiocoraline integrated by an attP mechanism.[4] PCR conditions were: 2 min at 94 °C; 30 cycles (each one including these steps: 30 sec at 94 °C, 1 min at 58 °C and 90 sec at 68 °C); an elongation step of 5 min at 68 °C; and a final step of 15 min at 4 °C.

The PCR product was purified from an agarose gel using a Gel Extraction Kit (QIAGEN), and cloned into pCR-Blunt plasmid vector, giving rise to the pGVR1 plasmid. The pGVR1 plasmid contains the

1,553-bp DNA fragment internal to *tioN* (confirmed by sequencing) flanked by *Xba*I and *Hind*III restriction sites (from pCR-Blunt polylinker). This *Xba*I-*Hind*III DNA fragment was then subcloned into the pMAS13 plasmid vector, generating pGVR7. pMAS13 is a pBluescript II SK derivative containing a thiostrepton resistance gene (used as a marker for easy selection of transformant colonies) cloned at the unique *Nae*I site (outside the polylinker region). It also contains sequence of the promoter *PermE,* cloned between *Xba*I/*Sac*I restriction sites, used to ensure transcription of the *tioM* neighboring gene. Therefore, after single cross-over on the *tioN* gene, and its corresponding knockout, transcription of the *tioM* gene is completely assured and no polar mutation effects are expected on that putative operon. pGVR7 was used for transformation of *S. albus*-pFL1049 protoplasts on R5 Petri dishes, following the classical method,[1] and after 24 h the plates were overlayed with H_2O containing thiostrepton with a final concentration of 50 μg thiostrepton/mL of R5. Three thiostrepton-resistant colonies were obtained after several transformation experiments with pGVR7.

One colony of *S. albus*-pFL1049-Δ*tioN* was inoculated in Bennet medium (with 50 μg thiostrepton/mL) for sporulation, the spores harvested and maintained in a 50% glycerol stock solution at -20 °C, and used for the production experiments. One million spores from this *tioN* mutant strain were inoculated in a flask containing TSB medium (25 mL), supplemented with 5 μg thiostrepton/mL, 0.75% glycine and $MgCl_2$ (5 mM). After incubation (30 °C, 250 rpm, 72 h) the cells were harvested by centrifugation (8,000 rpm) and used to obtain genomic DNA from this mutant colony using the Salting Out protocol.[1] The genomic DNA from *S. albus*-pFL1049, taken as control, and the genomic DNA from *S. albus*-pFL1049-Δ*tioN* were digested by *Sac*I and this digestion was used for testing whether *tioN* was effectively mutated in this thiostrepton-resistant transformant colony by Southern Blot hybridization, using the DNA fragment internal to the *tioN* gene sequence obtained from pGVR1 as a probe.

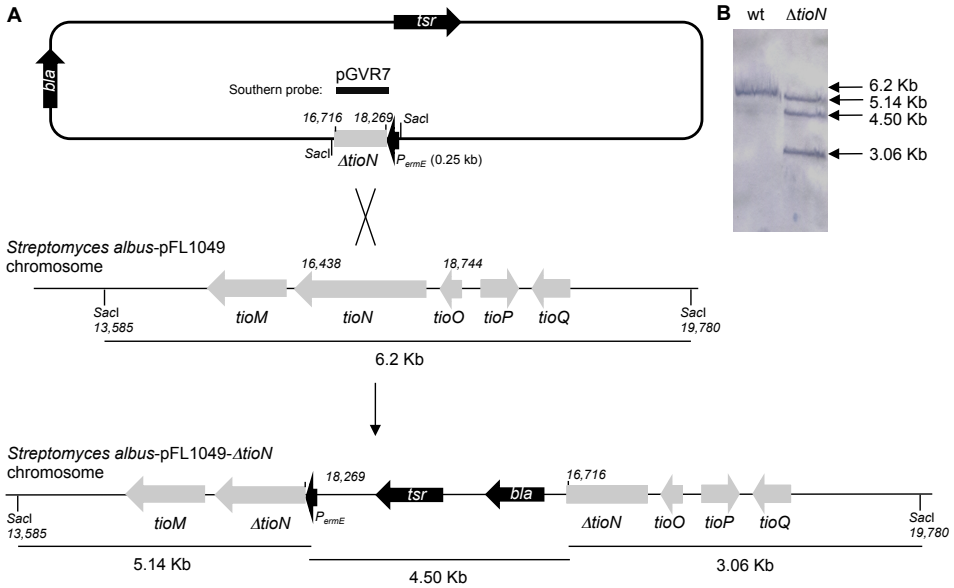

Fig. S1. Preparation of the *tioN* mutant strain (Δ*tioN*). **A.** Transformation of *S. albus*-pFL1049 strain with the plasmid pGVR7 renders a gene disruption mutant in the *tioN* gene after an integration event through the internal *tioN* region cloned into pGVR7. **B.** Southern blot hybridizations from wild-type (wt) and Δ*tioN* mutant strain chromosomal DNA confirming the gene disruption event in the *tioN* region. The probe for the Southern hybridization is in the internal *tioN* region cloned in pGVR7.

Generation of a *tioT* knockout in *S. albus*-pFL1049. The preparation of the *tioT* mutant strain (Δ*tioT*) is depicted in Fig. S2. A 2,990-bp DNA fragment upstream of *tioT* gene sequence was PCR-amplified using

oligonucleotides GVR-TL2-up (5'-AAAAGCAGCATCACCCGAAGAG-3') and GVR-TL2-rp (5'-AAAAAACGGGTTGACGCTCATCT-3'). The PCR reaction mixture and conditions were as above, except for 3 min instead of 90 sec at 68 °C.

The PCR product was purified from an agarose gel and cloned into pCR-Blunt plasmid vector to give the pGVR23 plasmid containing the 2,990-bp DNA fragment upstream to *tioT* (which was confirmed by sequencing) flanked by *Eco*RI restriction sites (from pCR-Blunt polylinker). This DNA fragment was then subcloned as an *Ecl*136II/*Eco*RV 2.9-kb DNA band into the pMAS13 plasmid vector digested with *Ecl*136II, generating pGVR26. A 2,993-bp DNA fragment downstream of *tioT* gene sequence was PCR-amplified using the oligonucleotides GVR-TR2-up (5'-AAAAGAGGAGAACTGGACCGACAT-3') and GVR-TR2-rp (5'-AAAACCAATTACGATTCCCAATACTCA-3'). PCR conditions were: 2 min at 94° C; 30 cycles (each one including these steps: 30 sec at 94° C, 1 min at 57° C and 3 min at 68° C); an elongation step of 5 min at 68° C; and a final step of 15 min at 4° C.

The PCR product was purified from an agarose gel and cloned into pCR-Blunt plasmid vector to give the pGVR24 plasmid containing the 2,993-bp DNA fragment downstream to *tioT* (which was confirmed by sequencing) flanked by *Eco*RI restriction sites (from pCR-Blunt polylinker). The *ermE* gene for erythromycin resistance was cloned from pNAE1 as a *Spe*I/*Eco*RV 1.7-kb DNA fragment into pCR-Blunt (digested with same enzymes) to give pGVR25. This *ermE* resistance gene was then subcloned from pGVR25 as a *Sac*I 1.7-kb DNA band into the pGVR26 plasmid vector digested with *Sac*I to generate pGVR27. In this pGVR27 plasmid construction, the *ermE* resistance gene is located downstream of the 2,990-bp DNA fragment upstream of the *tioT* gene, and the P_{ermE} promoter (originally from pMAS13) is placed downstream of the *ermE* gene. Finally, the 2,993-bp DNA fragment downstream of *tioT* gene cloned in pGVR24 was subcloned as a *Xba*I/*Spe*I 2.9-kb DNA fragment into pGVR27 (digested with *Xba*I) to give rise to pGVR28. In pGVR28, both regions originally flanking *tioT* gene, are now flanking the *ermE* gene, with an extra copy of P_{ermE} promoter downstream of this *ermE* gene, in order to avoid any stop in the transcription of *tioU,V* genes once the final mutant strain for *tioT* was generated.

pGVR28 was used for transformation of *S. albus*-pFL1049 protoplasts on R5 Petri dishes, following the classical method, and after 24 h the plates were overlayed with H_2O containing erythromycin to achieve a final concentration of 200 μg of erythromycin/mL of R5 medium. 284 erythromycin-resistant colonies were obtained after several transformation experiments with pGVR28. In these colonies, the pGVR28 plasmid had been integrated by homologous recombination at least at one of the *tioT* flanking regions. From all these colonies, only five showed a thiostrepton-sensitive phenotype when plated on R5 Petri dishes containing 50 μg of thiostrepton/mL R5 medium. These 5 colonies had suffered two homologous recombination events, each one at each of the *tioT* flanking regions present in pGVR28. In these mutant colonies, the *tioT* gene has been replaced by the *ermE* gene, including a downstream copy of the *ermE* gene promoter, which assures transcription of *tioU,V* genes in this mutant chromosome.

One colony of *S. albus*-pFL1049-D*tioT* (colony T17) was inoculated for sporulation in Bennet medium (with 200 μg of erythromycin/mL), and the spores harvested and maintained in a 50% glycerol stock solution at -20 °C, and used for the production experiments. One million spores from this *tioT* mutant strain were inoculated in a flask containing TSB medium (25 mL), supplemented with 200 μg of erythromcyin/mL, 0.75% glycine, and $MgCl_2$ (5 mM). After incubation (30 °C, 250 rpm, 72 h) the cells were harvested by centrifugation (8,000 rpm) and used to obtain genomic DNA from this T17 mutant colony using the Salting Out protocol.[1] The genomic DNA from *S. albus*-pFL1049 control strain and genomic DNA from *S. albus*-pFL1049-Δ*tioT* were digested with *Sph*I and this digestion of DNA was used for testing that *tioT* was effectively mutated in this erythromycin-resistance and thiostrepton-sensitive transformant colony by Southern Blot hybridization, using the 2.9-kb DNA fragment cloned in pGVR23 as a probe.

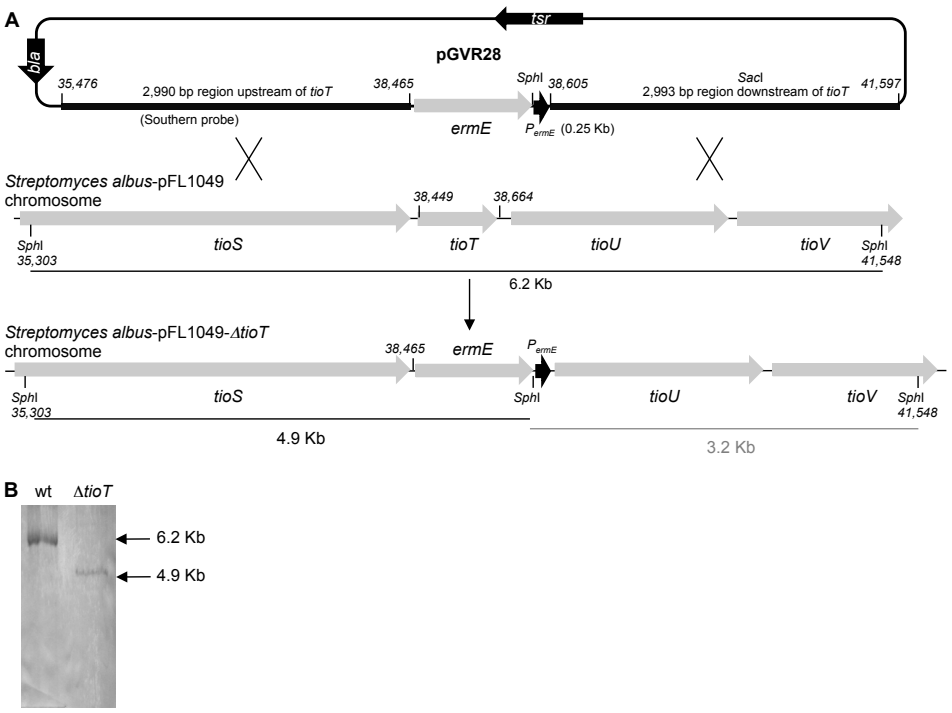

Fig. S2. Preparation of the *tioT* mutant strain (Δ*tioT*). **A.** Transformation of *S. albus*-pFL1049 strain with the plasmid pGVR28 renders a gene disruption mutant in the *tioT* gene after two homologous recombination events through the *tioT* flanking regions cloned into pGVR28. **B.** Southern blot hybridizations from wild-type (wt) and Δ*tioT* mutant strain chromosomal DNA confirming the gene disruption event in the *tioT* region. The probe for the Southern hybridization is in the 2,990 region upstream from *tioT* (35,476-38,465).

Monitoring of thiocoraline production in *S. albus*-pFL1049, *S. albus*-pFL1049-Δ*tioN*, and *S. albus*-pFL1049-Δ*tioT*. To establish if the *S. albus*-pFL1049-Δ*tioN* and *S. albus*-pFL1049-Δ*tioT* clones can produce thiocoraline, R5A medium (25 mL) supplemented with thiostrepton (5 μg/mL) (for Δ*tioN*) or with erythromycin (200 μg/mL) (for Δ*tioT*) was inoculated with spores of these mutant strains. After incubation (30 °C, 250 rpm, 4 days), the culture was extracted with two volumes of EtOAc (2×25 mL), and the organic solvent removed in a speed vac. The dry extract was resuspended in a 1:1/MeOH:DMSO mixture (1 mL) and analyzed by RP-HPLC (injection volume: 10 μL; flow rate: 0.5 mL/min; solvents used: A = H_2O (0.1% acetic acid) (for Δ*tioN*) or H_2O (0.1% TFA) (for Δ*tioT*), B = MeCN; gradient used = 10%B for 10 min, 10-99%B over 20 min, 99%B for 10 min, and 10%B for 10 min) (Figs. S3 (for Δ*tioN*) and S4 (for Δ*tioT*)) and ESI-MS. Product elution was monitored at 230 nm. The retention time for thiocoraline under these conditions was 25.8 min (for Δ*tioN* experiments) and 29.1 (for Δ*tioT* experiments). The production of thiocoraline in the *S. albus*-pFL1049 control strain was confirmed by ESI-MS in the positive ion mode with a capillary voltage of 3 kV and a cone voltage of 20 V [electrospray ionization for thiocoraline ($C_{48}H_{56}N_{10}O_{12}S_6$): *m/z* calcd., 1157.4; obs., 1157.32]. No thiocoraline nor thiocoraline intermediates were present in the extract from the *S. albus*-pFL1049-Δ*tioN* or *S. albus*-pFL1049-Δ*tioT* mutant strains (Figs. S3 and S4).

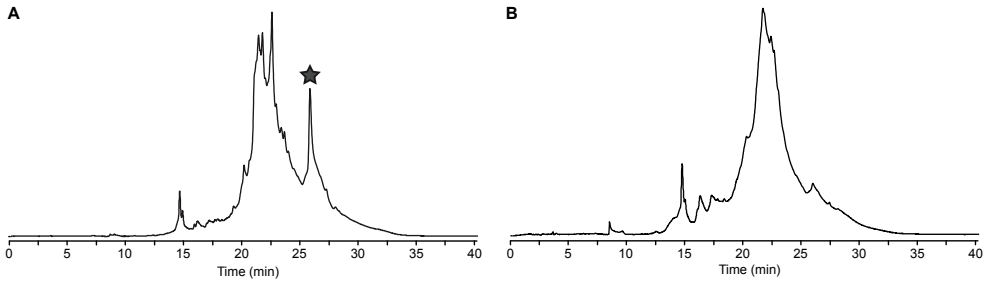

Fig. S3. HPLC chromatograms (with the thiocoraline peak at 25.8 min indicated by a star) of extracts from cultures of strains **A.** *S. albus*-pFL1049 and **B.** *S. albus*-pFL1049-Δ*tioN*. No thiocoraline production is detected in cultures of *S. albus*-pFL1049-Δ*tioN*.

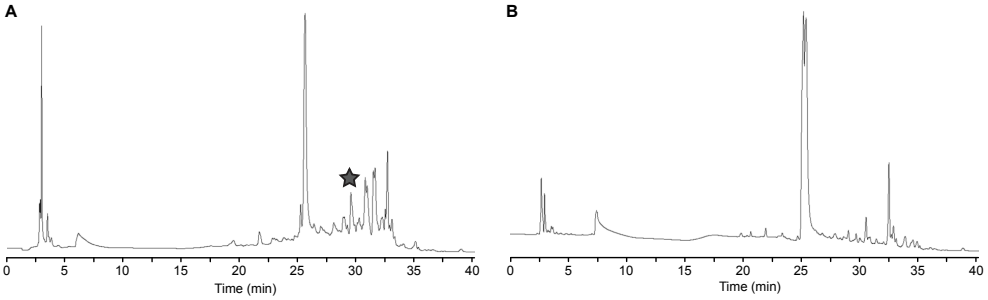

Fig. S4. HPLC chromatograms (with the thiocoraline peak at 29.1 min indicated by a star) of extracts from cultures of strains **A.** *S. albus*-pFL1049 and **B.** *S. albus*-pFL1049-Δ*tioT*. No thiocoraline production is detected in cultures of *S. albus*-pFL1049-Δ*tioT*.

Preparation of pTioN-pET28a and pTioT-pACYCDuet overexpression constructs. The forward and reverse primers (5'-TCTCCGCATATGCCCTGGAGCGTATGGAACCAG-3') and (5'-CCACAGGAATTCTCAGAGCCGGCTGAGCAGCG-3') containing *Nde*I and *Eco*RI restriction sites (underlined), respectively, were used for amplification of the *tioN* gene. The forward and reverse primers (5'-GGATCCGAATTCGATGAGCGTCAACCCGTTCGATG-3') and (5'-AGCCCAAAGCTTTCATGCTGCCGACATC-3') containing *Eco*RI and *Hind*III restriction sites (underlined), respectively, were used for amplification of the *tioT* gene. PCR reactions were carried out using *Micromonospora* sp. ML1 genomic DNA.[5] The amplified *tioN* and *tioT* fragments were digested and subcloned into the linearized pET28a and pACYCDuet-1 vectors *via* the corresponding *Nde*I/*Eco*RI and *Eco*RI/*Hind*III, respectively. All cloning experiments were performed in *E. coli* TOP10 cells. The DNA sequences of expression clones pTioN-pET28a, and pTioT-pACYCDuet-1 were confirmed by DNA sequencing (University of Michigan DNA Sequencing Core) (Accession numbers CAJ34370 (*tioN*) and CAJ34376 (*tioT*)).

Co-overexpression and purification of TioN and TioT proteins. The purified plasmid pTioN-pET28a was co-transformed with pTioT-pACYCDuet-1 into chemically competent *E. coli* BL21(DE3)*ybdZ*::*aac(3)IV* cells for protein co-overexpression and purification. The proteins were grown in LB medium (5×1 L) supplemented with kanamycin (50 μg/mL), chloramphenicol (25 μg/mL), and $MgCl_2$ (10 mM final concentration). An overnight culture (4 mL) prepared from fresh transformants was used to inoculate each L of LB broth. The cells were grown (28 °C, 200 rpm) to an attenuance at 600 nm of 0.5, at which point they were induced with IPTG (0.1 mM final concentration). After an additional 16-18 h of growth (20 °C, 200 rpm) the cells were harvested by centrifugation (6,000 rpm, 4 °C, 5 min) and resuspended in buffer A (25 mM Tris pH 8.0, 400 mM NaCl, 10% glycerol). The resuspended cells were

lysed by sonication (5 min using 10 s "on" alternating with 20 s "off") and the cell debris was removed by centrifugation (16,000 rpm, 4 °C, 45 min,). Imidazole (2 mM final concentration) was added to the supernatant before incubation (4 °C, 2 h with gentle rocking) with Ni-NTA agarose resin (3 mL; Qiagen). The resin was then loaded onto a column and washed with buffer A containing imidazole (2 mM (10 mL), 5 mM (10 mL), 20, 40, and 60 mM (5 mL each), as well as 200 and 500 mM (2×5 mL each)). As determined by SDS-PAGE, fractions containing the pure desired protein were combined and dialyzed (4 °C, 14-18 h total) against a total of 6 L of buffer B (40 mM Tris pH 8.0, 200 mM NaCl, 10% glycerol). The TioN protein (co-purified with TioT) was concentrated, flash frozen, and stored at -80 °C (Fig. S5). Protein concentration was determined using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific). Protein yields for TioN when co-overexpressed with TioT was about 3-5 mg/L of bacterial culture.

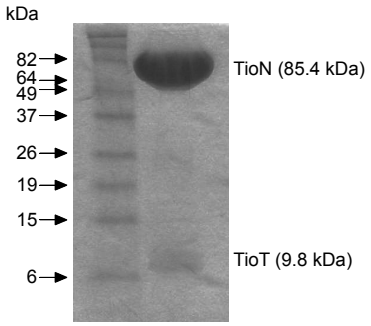

Fig. S5. Coomasie blue-stained 15% Tris-HCl SDS-PAGE gel showing the TioN protein (co-purified with TioT). 6 μg of protein was loaded on the gel.

Substrate specificity and determination of kinetic parameters for TioN(A domain) by ATP-[^{32}P]PP$_i$ exchange assays. To decipher between steps a_1 and b_2 in Fig. 2, we investigated the substrate specificity of the TioN(A domain). ATP-[^{32}P]PP$_i$ exchange assays were performed at rt in reactions (100 μL) containing Tris-HCl (75 mM, pH 7.5), $MgCl_2$ (10 mM), TCEP (5 mM, pH 7.0), ATP (5 mM), amino acid substrate (5 mM), and $Na_4P_2O_7$ (1 mM, spiked with 400,000 cpm of [^{32}P]PP$_i$ per reaction). The reactions were initiated by addition of TioN (2.5 μM) and incubated for 2 h prior to quenching with charcoal suspensions (500 μL) [1.6% (w/v) activated charcoal, 4.5% (w/v) $Na_4P_2O_7$, and 3.5% (v/v) perchloric acid in H_2O]. The charcoal was pelleted by centrifugation (13,000 rpm, rt, 7 min), washed twice with a wash solution (500 μL) [4.5% (w/v) $Na_4P_2O_7$, and 3.5% (v/v) perchloric acid in H_2O], resuspended in H_2O (500 μL), and counted by liquid scintillation counting (Fig. 3A). For the determination of the kinetic parameters (K_m and k_{cat}) for L-Cys, *S*-Me-L-Cys, L-Val, L-Leu, and L-Ile reactions were performed at rt with varying concentrations of amino acid (0.05, 0.1, 0.25, 0.5, 1, 1.75, 2.5, 5, and 10 mM). The reactions were started by addition of TioN (2.5 μM) and stopped after 15 min. The experiments were carried out in duplicates for each substrate concentration (Fig. 3B and Table 1).

Investigation and comparison of steps a_1-a_2 and c_1-c_3 by TCA precipitation assays. To compare the loading of L-Cys-AMP (steps a_1-a_2 in Fig. 2) to that of *S*-Me-L-Cys-AMP (steps c_1-c_3) onto TioS(T_4), time course TCA precipitation assays were performed using [^{35}S]L-Cys (Fig. 4A). All assays (including those of the next section below) required the preparation, in 2 h, of the TioS(T_4) holo (active) enzyme (mixture #1, 12.5 μL for each reaction time point) in reactions containing Tris-HCl (75 mM, pH 7.5), $MgCl_2$ (10 mM), TCEP (1 mM, pH 7.0 adjusted at rt), CoA (500 μM), apo (inactive) TioS(T_4) (440 μM), and Sfp (1 μM). For the investigation of steps a_1-a_2 (activation of L-Cys to L-Cys-AMP (a_1), followed by loading onto holo TioS(T_4) (a_2)), simultaneously to holo TioS(T_4) preparation, the activation of [^{35}S]L-Cys to [^{35}S]L-Cys-AMP was performed in a separate reaction mixture #2 (12.5 μL for each reaction time point)

containing Tris-HCl (75 mM, pH 7.5), $MgCl_2$ (10 mM), TCEP (1 mM, pH 7.0), ATP (5 mM), TioN (2.5 μM), co-expressed and co-purified with TioT, L-Cys (3 mM), and [^{35}S]L-Cys (containing 5,000,000 cpm per reaction). After 2 h of incubation at rt, mixtures #1 and #2 (12.5 μL each) containing the holo enzyme TioS(T_4) (mixture #1) and the [^{35}S]L-Cys-AMP (mixture #2), were combined by the addition of mixture #2 to #1. The resulting reaction mixture (25 μL, for each reaction time point) was quenched with 10% TCA (100 μL) after 0, 3, 6, 10, 20, 30, 60 and 120 min. These reaction time points were the same for all radioactive experiments in this section and in the experimental section below. For time point zero, 12.5 μL of each mixture (#1 and #2) were taken and directly added to a 100 μL of 10% TCA. The precipitated protein was pelleted by centrifugation (13,000 rpm, rt, 7 min), washed with 10% TCA (100 μL), and resuspended in 88% formic acid (100 μL). The radiolabeled product was counted by liquid scintillation counting. For the investigation of steps c_1-c_3 (activation of L-Cys to L-Cys-AMP (step c_1), followed by *S*-methylation to *S*-Me-L-Cys-AMP (step c_2), and then loading onto holo TioS(T_4) (step c_2)) using [^{35}S]L-Cys, mixture #2 was incubated first for 2 h, then an additional 2.5 μM of TioN and 5 mM of SAM were added (the total volume of mixture #2 was 12.5 μL per reaction time point after additional TioN and SAM), and the mixture was allowed to incubate for additional 2 h at rt. Simultaneously, mixture #1 (for the preparation of holo TioST4, 12.5 μL per reaction time point) was incubated for 2 h at rt. The two mixtures were then combined by adding mixture #2 to #1 to start the [^{35}S]L-Cys loading time course. Sample processing, and scintillation counting was performed as described above.

Investigation of order of methylation by TioN(M domain) by comparison of steps a_1-a_3 and c_1-c_3 by TCA precipitation assays. To verify if methylation by TioN(M domain) can occur prior (steps c_1-c_3 in Fig. 2) and/or after (steps a_1-a_3) loading of L-Cys onto TioS(T_4), time course TCA precipitation assays were performed using [methyl-^{3}H]SAM (Fig. 4B). To investigate steps c_1-c_3 in Fig. 2 using [methyl-^{3}H]SAM, mixture #2 (described in the above experimental section) was modified to exclude [^{35}S]L-Cys (replaced by H_2O). After 2 h incubation of mixture #2 at rt, an additional 2.5 μM of TioN, 5 mM of SAM, and [methyl-^{3}H]SAM (containing 3,500,000 cpm per reaction) were added (total volume was 12.5 μL per reaction time point) and the mixture was allowed to incubate for additional 2 h. Simultaneously, the holo TioS(T_4) mixture #1 was prepared as above and the loading time course was initiated by combining the two mixtures (by adding mixture #2 to #1). Sample processing and scintillation counting was performed as described above. Finally, for the investigation of steps a_1-a_3 in Fig. 2 using [methyl-^{3}H]SAM, mixture #2 for the activation of L-Cys to L-Cys-AMP (step a_1) containing 3 mM L-Cys and no [^{35}S]L-Cys was incubated for 2 h. Simultaneously, the preparation of the holo TioS(T_4) mixture #1 was performed as described above and incubated for 2 h. The two mixtures were then combined by addition of mixture #2 to #1 and incubated for additional 2 h at rt to allow loading of L-Cys-AMP onto holo TioS(T_4) (step a_2). Then, an additional 2.5 μM of TioN, 5 mM of SAM, and [methyl-^{3}H]SAM (containing 3,500,000 cpm per reaction) were added (making total volume to 25 μL per reaction time point) to start methylation of L-Cys-*S*-TioS(T_4) time course (step a_3). Processing and counting of time point samples was performed as described above.

References

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