

Supporting Information

Deciphering Nature's intricate way of *N,S*-dimethylating L-cysteine: Sequential action of two bifunctional adenylation domains

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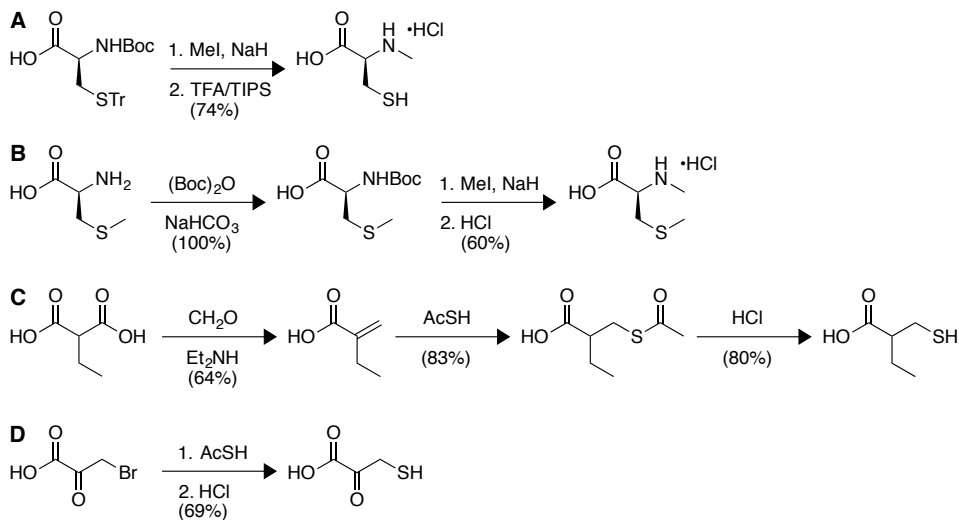


Fig. S1. Synthetic schemes for the preparation of the non-commercially available compounds used to determine the substrate profile of the enzymes used in this study as well as the ability of these enzyme to methylate the compounds found to be substrates.

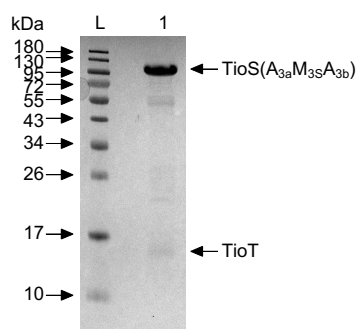


Fig. S2. Coomassie blue-stained 15% Tris-HCl SDS-PAGE gel showing the TioS(A_{3a}M_{3S}A_{3b}) (lane 1, 105.5 kDa) protein (co-purified with TioT). 10 µg of the protein was loaded on the gel. L indicates the PageRuler™ Prestained Protein Ladder, 10 to 180 kDa from Thermo Scientific.

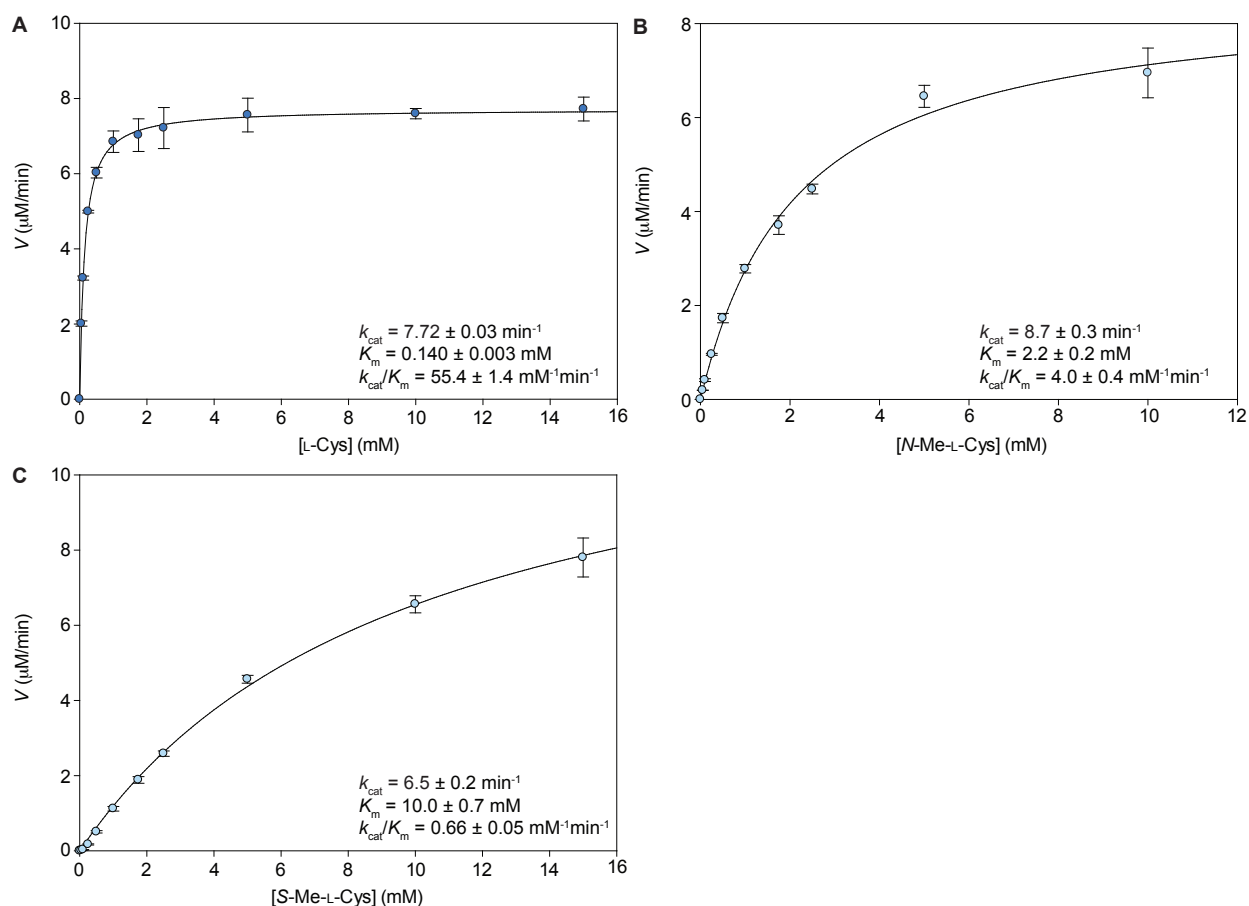


Fig. S3. Michaelis-Menten kinetic parameters of the TioS(A_{3a}M_{3S}A_{3b})-catalyzed adenylation of **A.** L-Cys, **B.** N-Me-L-Cys, and **C.** S-Me-L-Cys.

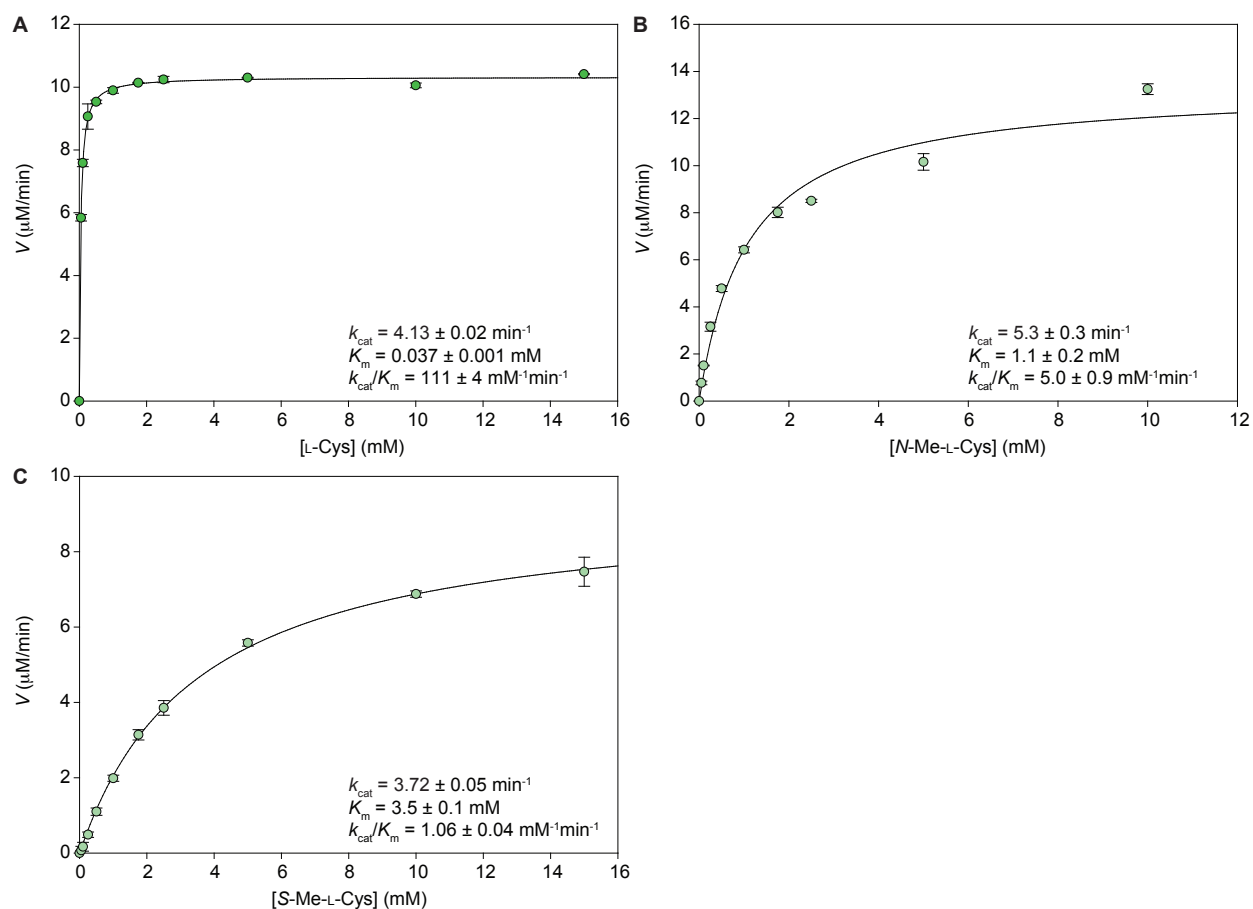


Fig. S4. Michaelis-Menten kinetic parameters of the TioN(A_aM_NA_b)-catalyzed adenylation of **A.** L-Cys, **B.** N-Me-L-Cys, and **C.** S-Me-L-Cys.

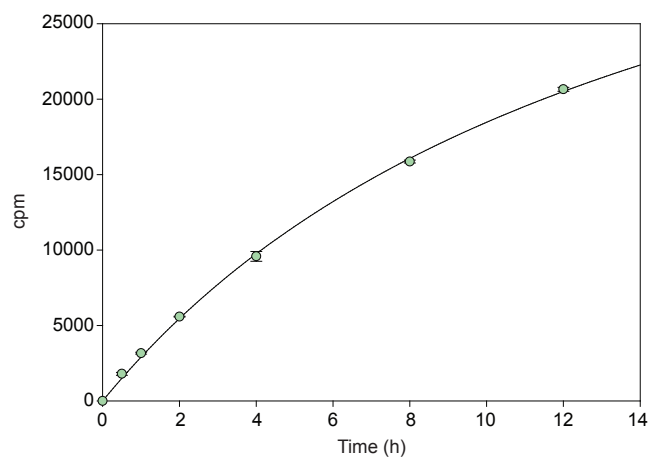


Fig. S5. Adenylation of N,S-diMe-L-Cys by TioN(A_aM_NA_b) observed in a time course assay by using ATP-[³²P]PP_i exchange assay.

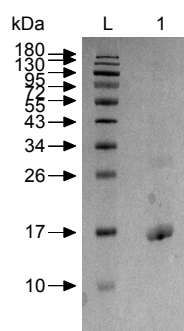


Fig. S6. Coomassie blue-stained 15% Tris-HCl SDS-PAGE gel showing the TioS(T₃) (lane 1, 13.9 kDa) protein. 6 µg of protein was loaded on the gel. L indicates PageRuler™ Prestained Protein Ladder, 10 to 180 kDa from Thermo Scientific.

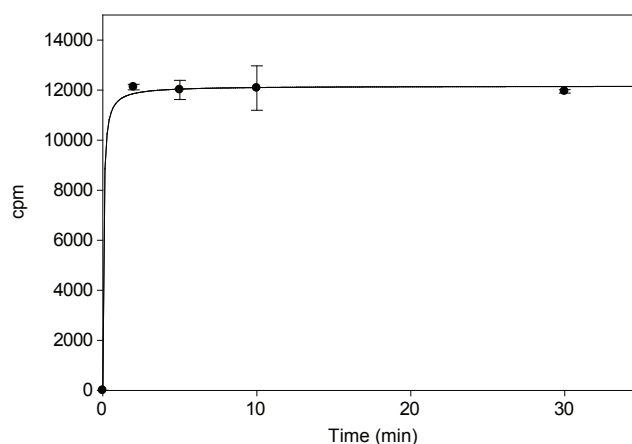


Fig. S7. Monitoring of the conversion of apo to [³H]acetyl-S-TioS(T₃) observed by using a TCA precipitation assay using [³H]AcCoA.

Table S1. Primers used in this study.

Primer #	Primer name	Primer sequence	5' or 3' primer
1	<i>tioS(A_{3a}M_{3S}A_{3b})-fwd</i>	GAGCAGCATATGGAGAGCCCGACCTCGGCGTC	5'
2	<i>tioS(A_{3a}M_{3S}A_{3b})-rev</i>	ACTGGCAAGCTTTTACTCCGGCACCGGCAGCGC	3'
3	<i>tioS(T₃)-fwd</i>	GACCGGCATATGCTGCCGGTGCCGGAGTACG	5'
4	<i>tioS(T₃)-rev</i>	CCGTGAAGCTTTTAGGAAACCGGGAGCCGGTCCG	3'

^a The introduced restriction sites are underlined for each relevant primers with the 5' primers introducing a *Nde*I restriction site and the 3' primers introducing a *Hind*III restriction site. ^b Both constructs encode a NHis₆-tagged protein.