A novel [3Fe-4S] cluster and tRNA dependent aminoacyltransferase BlsK in the biosynthesis of Blasticidin S

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Supporting Information

Table S1 Primer list of this study

Name	Primer sequence	Note
tar-K-F	CCGATCGCAACACCAAGCCAGACAGAGGTCA CCCAGATGATTCCGGGGATCCGTCGACC	*blsK* PCR-targeting
tar-K-R	CTGTCCTCCTCGGGACGGGAAGGAGGCTGAC GGCTGTCATGTAGGCTGGAGCTGCTTC	
CON-K-F	GCCAGACAGAGGTCACCCAG	WXK3 verification
CON-K-R	GGGAAGGAGGCTGACGGCTG	
139-K-F	GGAATTCCATATGATGCAGTCCGCCCACACCG A	*blsK* compelement
139-K-R	CGGGATCCTCAGCCTCGCCGGGGAACGACTC	
blsK-F	GGAATTCCATATGATGCAGTCCGCCCACACCG A	*blsK* overexpression
blsK-R	CCGAATTCTCAGTGGTGGTGGTGGTGGTGGC CTCGCCGGGGAACGACTC	
Leu-F	TAATACGACTCACTATAGTCCGGGTGGCGGAA TGG	$tRNA^{leu}$ (CUC)
Leu-R	TGGTGTCCGAGGGGGGACTTGAACC	
C-S1F	GCGAGTTCACCATCAGCGGGCTCAACGACA	C236S
C-S1R	TGTCGTTGAGCCCGCTGATGGTGAACTCGC	
C-S2F	TCCTCGGCCCGCGCAGCGCCTACGGCCTGG	C253S
C-S2R	CCAGGCCGTAGGCGCTGCGCGGGCCGAGGA	
C-S3F	CCTACGGCCTGGCCAGCTACAAGCCCGAGG	C259S
C-S3R	CCTCGGGCTTGTAGCTGGCCAGGCCGTAGG	
C-S4F	TCGTCCTCAGCGCCAGCAACAGCGGGCCGC	C282S
C-S4R	GCGGCCCGCTGTTGCTGGCGCTGAGGACGA	
C-A4F	GCCGAAGTCGTCCTCAGCGCCGCCAACAGC	C282A
C-A4R	GTCGGACAGCGGCCCGCTGTTGGCGGCGCT	

C-S5F	TCGACGAGGTGACGAGCCACTGCGGCCGGC	C480S
C-S5R	GCCGGCCGCAGTGGCTCGTCACCTCGTCGA	
C-S6F	AGGTGACGTGCCACAGCGGCCGGCCCGCGC	C482S
C-S6R	GCGCGGGCCGGCCGCTGTGGCACGTCACCT	
C-S7F	TCCTGGACACGGTGAGCGTGGTGTGTCTGC	C503S
C-S7R	GCAGACACACCACGCTCACCGTGTCCAGGA	
C-S8F	CGGTGTGCGTGGTGAGTCTGCGCTGCGGCG	C506S
C-S8R	CGCCGCAGCGCAGACTCACCACGCACACCG	
C-S9F	TGGTGTGTCTGCGCAGCGGCGATGTGACCT	C509S
C-S9R	AGGTCACATCGCCGCTGCGCAGACACACCA	

Table S2 Main strains and plasmids used in this study

Strains	**Relevant properties**	**Source**
Streptomyces lividans WJ2	Blasticidin S heterologous expression strain	1
Streptomyces lividans WXK3	Gene *blsK* inactivated mutant strain	This study
Streptomyces lividans WXK4	Gene *blsK* complement strain of WXK3	This study
Escherichia coli BL21 (DE3, Δ*pepN*)	AminopeptidaseN(*pepN*) inactivated strain	This study
Escherichia coli ET12567 /pUZ8002	*rec*F, *dam*, *dcm*, *hsdS*, Cml^r, Str^r, Tet^r, Km^r	2
Escherichia coli BW25113/pIJ790	*RepA101(ts), araBp-gam-be-exo, AraC, RepA101(ts)* Cml^r	2
Plasmids		
pJTU1780	Contain 35 kb BS biosynthetic cluster	1
pIJ778	*aadA* from Ω-fragment ($Spec^R$, $Strep^R$) + *oriT*	3
pIB139	*attP, Int, oriT, PermE*, aac(3)IV*	4
pET44b-*blsK*	*blsK* overexpression	This study
pET44b-*leuRS*	*leuRS* overexpression	This study

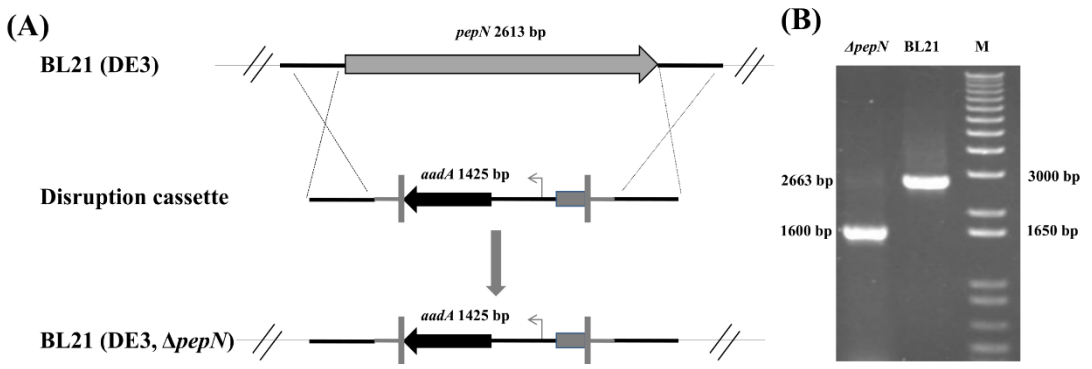

Figure S1. Schematic diagram of deletion of *pepN* in BL21 (DE3) strain (A) and PCR verification of the *pepN* deletion strain (B).

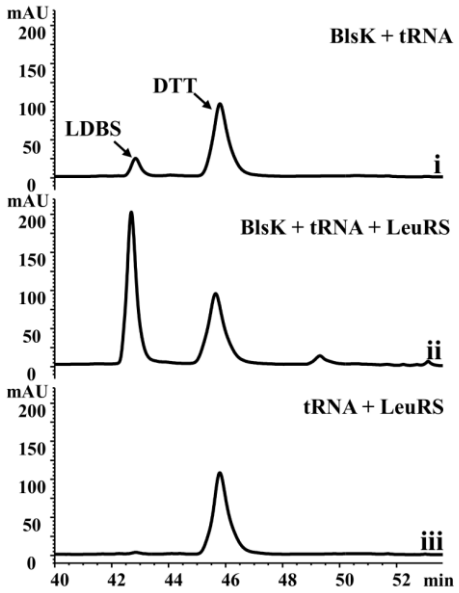

Figure S2. LeuRS is an auxiliary protein for BlsK function: (i) Ni-NTA purified BlsK incubated with DBS and *E.coli* total tRNAs; (ii) Ni-NTA purified BlsK incubated with DBS, *E.coli* total tRNAs, and LeuRS; (iii) LeuRS incubated with DBS and *E.coli* total tRNAs. 1 mM ATP, 10 mM Leu, 250 μM DBS, 1 mg/mL tRNAs, 20 mM $MgCl_2$, 10 mM KCl, and 10 mM DTT were added in the reaction mixture.

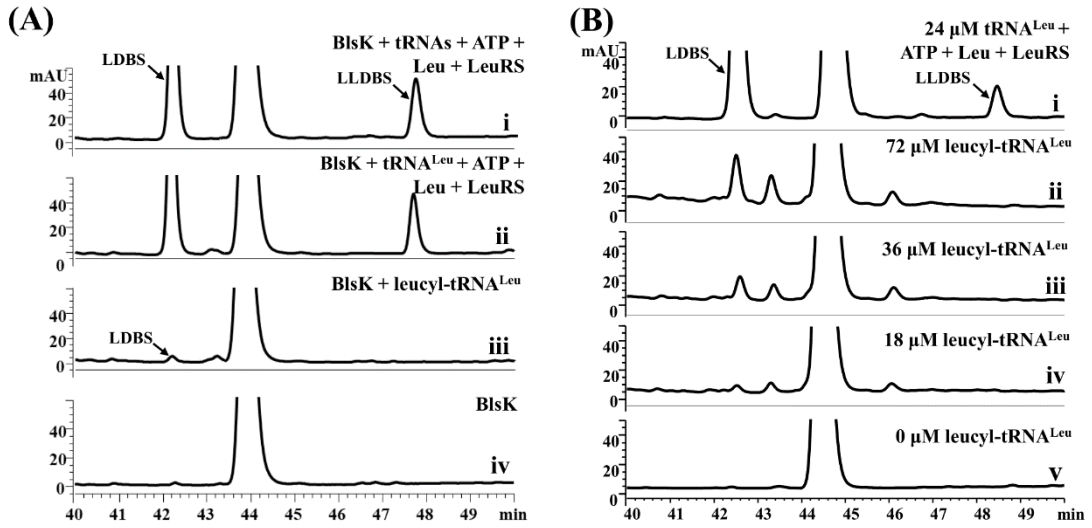

Figure S3. leucyl-tRNA^{Leu} is a direct substrate for BlsK. (A) The catalytic activity of BlsK with different substrates: (i) BlsK incubated with DBS, LeuRS, ATP, leucine, and mixed tRNAs; (ii) BlsK incubated with DBS, LeuRS, ATP, leucine, and *in vitro* prepared tRNA^{Leu}; (iii) BlsK incubated with DBS and *in vitro* prepared leucyl-tRNA^{Leu}; (iv) BlsK incubated with DBS as negative control. (B) The amount of LDBS produced is proportional to the amount of leucyl-tRNA^{Leu}: (i) BlsK incubated with DBS, LeuRS, ATP, leucine, and *in vitro* prepared tRNA^{Leu} as a positive control; (ii-v) BlsK incubated with DBS and different concentrations (ii: 72 μM; iii: 36 μM; iv: 18 μM; v: 0) of *in vitro* prepared leucyl-tRNA^{Leu}. DTT was added in all experiments.

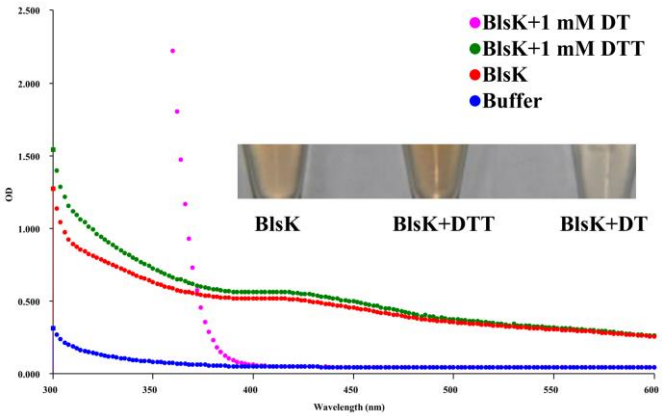

Figure S4. DTT darkens the color of BlsK slightly and increases the UV-visible absorption while addition of sodium dithionite (DT) bleaches the brownish color of BlsK and abolishes the absorption around 420 nM.

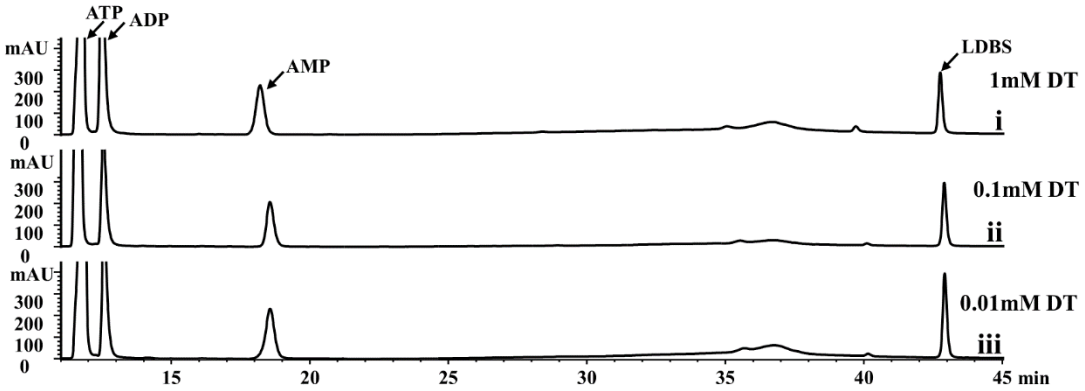

Figure S5. Dithionite (DT) does not affect the activity of BlsK: BlsK incubated with DBS, LeuRS, ATP, leucine, mixed tRNAs, and 1 mM (i), 0.1 mM (ii), 0.01 mM DT (iii), respectively.

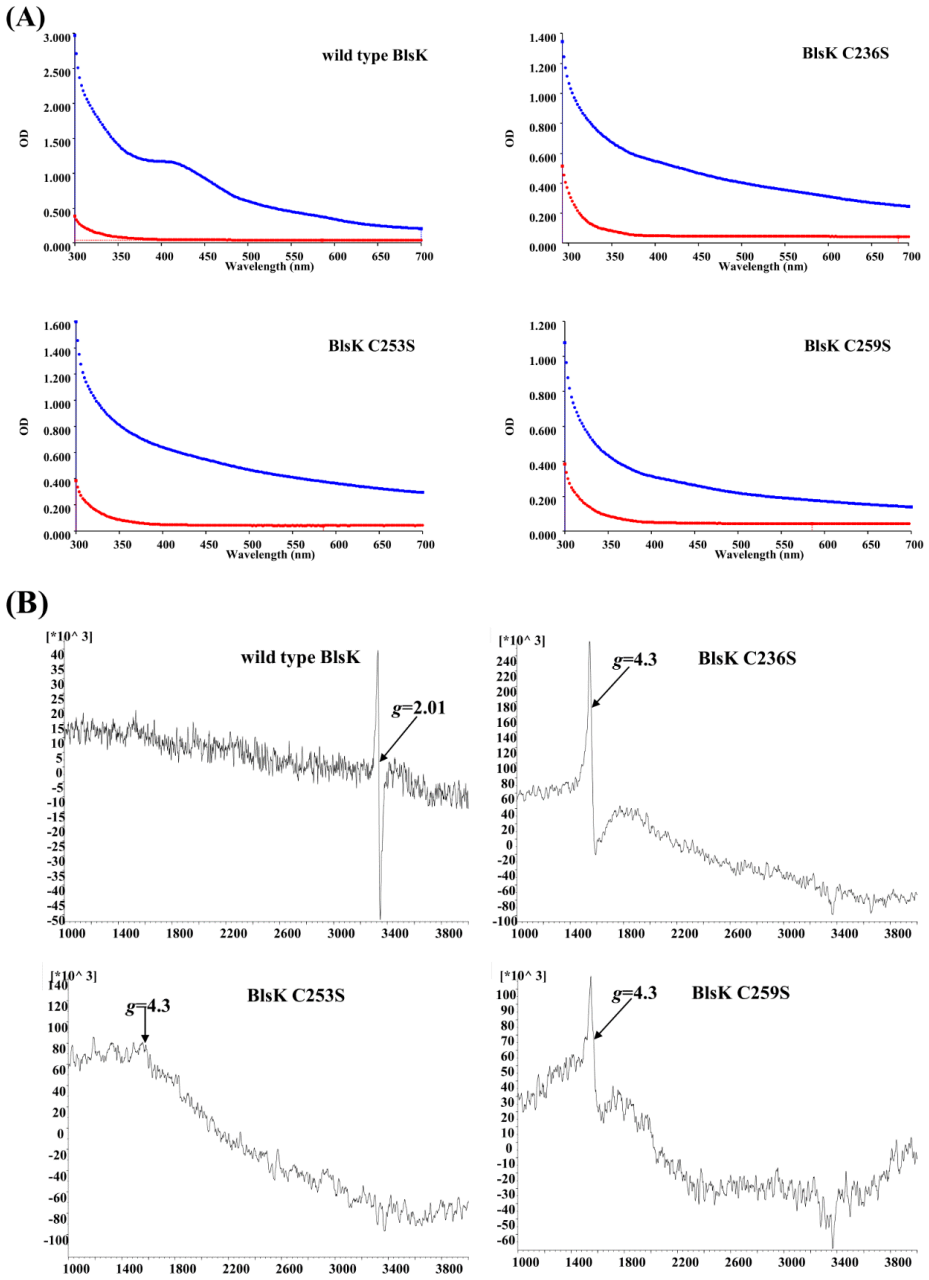

Figure S6. UV-visible spectra (A) and EPR analysis (B) of wild type, C236S, C253S, and C259S mutants of BlsK.

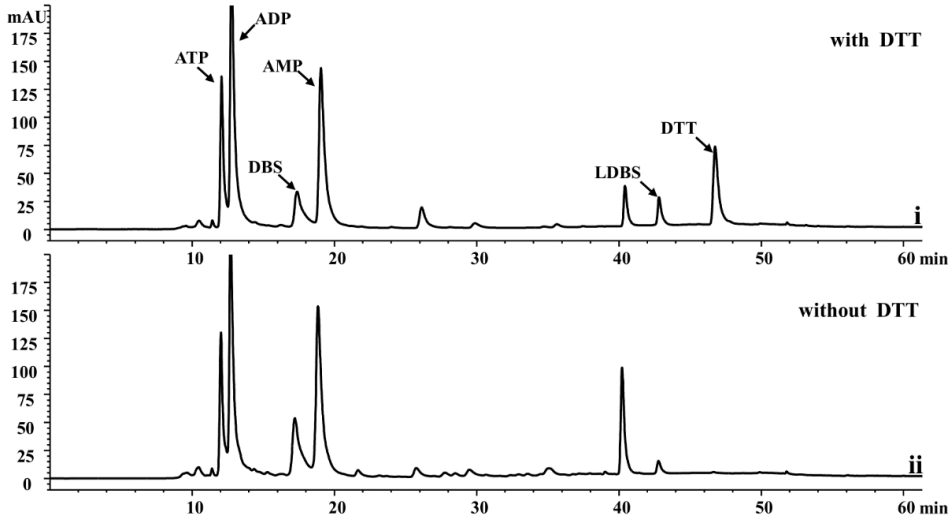

Figure S7. HPLC analysis of the effect of DTT on the activity of aerobically purified BlsK.

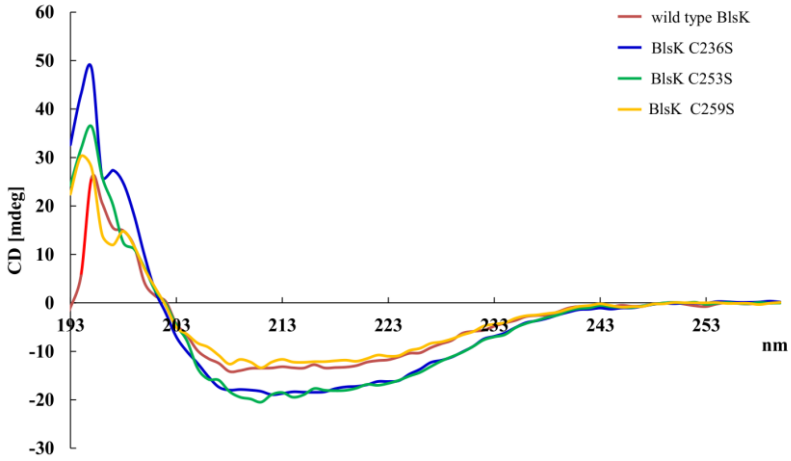

Figure S8. Circular dichroism analysis of wild type BlsK and three BlsK mutant proteins (C236S, C253S, and C259S).

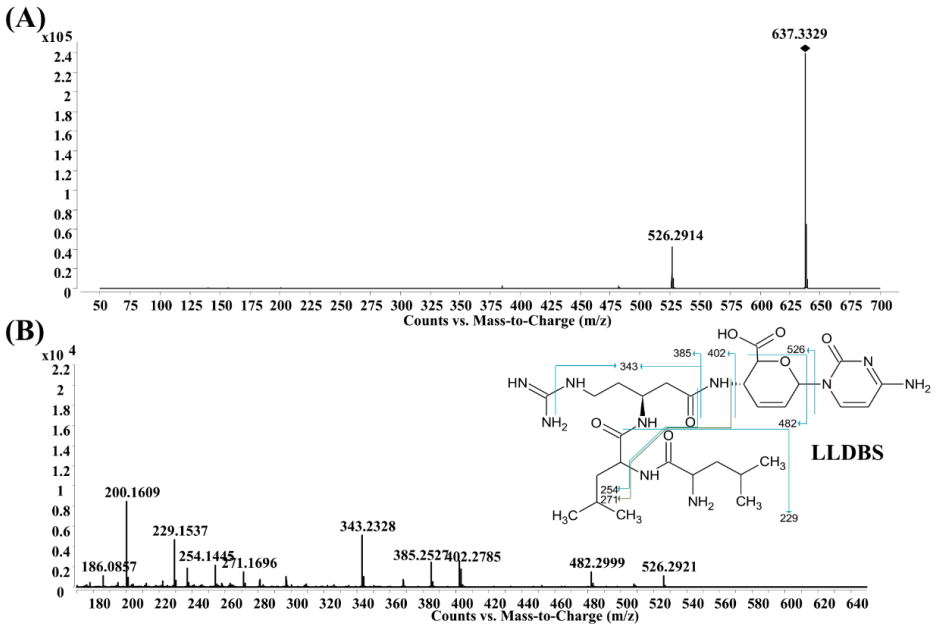

Figure S9. High-resolution mass spectrometric analysis of the LLDBS: (A) MS; (B) MS/MS. All secondary fragment peaks are consistent with LLDBS. The leucine used in the reaction was labeled at the α-carbon with ^{13}C isotope.

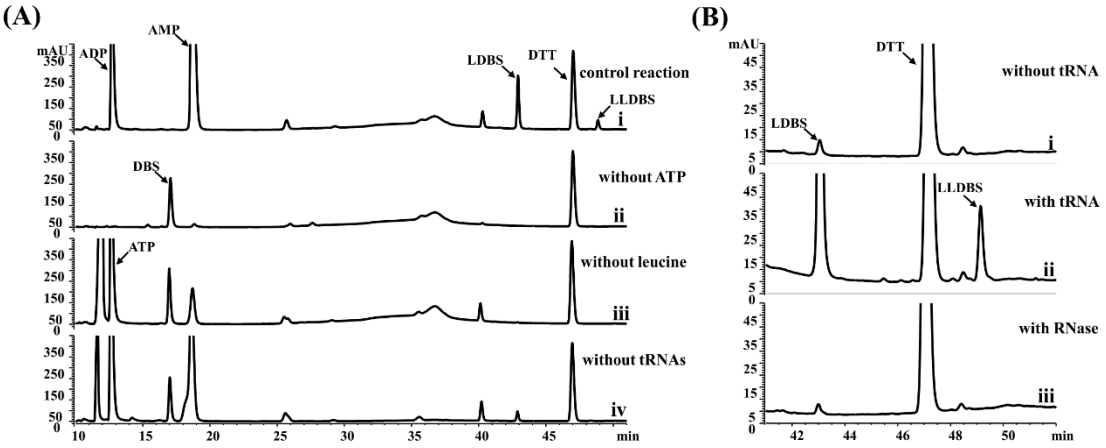

Figure S10. BlsK can also use leucyl-AMP as the substrate. (A) Analysis of precursors required by BlsK: (i) BlsK incubated with DBS, LeuRS, ATP, leucine, and $tRNA^{Leu}$; (ii) BlsK incubated with DBS, LeuRS, leucine, and $tRNA^{Leu}$ (no ATP); (iii) BlsK incubated with DBS, LeuRS, ATP, and $tRNA^{Leu}$ (no leucine); (iv) BlsK incubated with DBS, LeuRS, ATP, and leucine. (B) RNase A treatment did not inactivate BlsK: (i) BlsK incubated with DBS, LeuRS, ATP, and leucine; (ii) BlsK incubated with DBS, LeuRS, ATP, leucine, and $tRNA^{Leu}$; (iii) BlsK incubated with DBS, LeuRS, ATP, leucine, and RNase A.

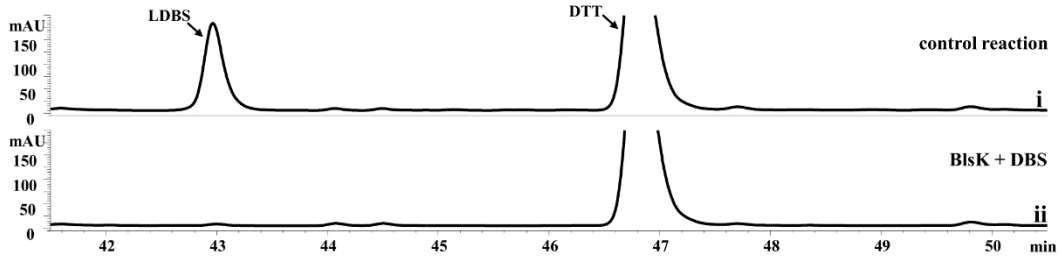

Figure S11. Purified BlsK does not have an attached leucyl group: (i) the normal reaction of BlsK with DBS, ATP, leucine, tRNA, and LeuRS as a positive control; (ii) BlsK (212 μM) and mixed with DBS (250 μM). 50 mM Tris-HCl (pH 8.0, 50 mM pH, 8.5, 100mM NaCl, 20 mM $MgCl_2$, 10 mM KCl, 10 mM DTT) was used as assay buffer.

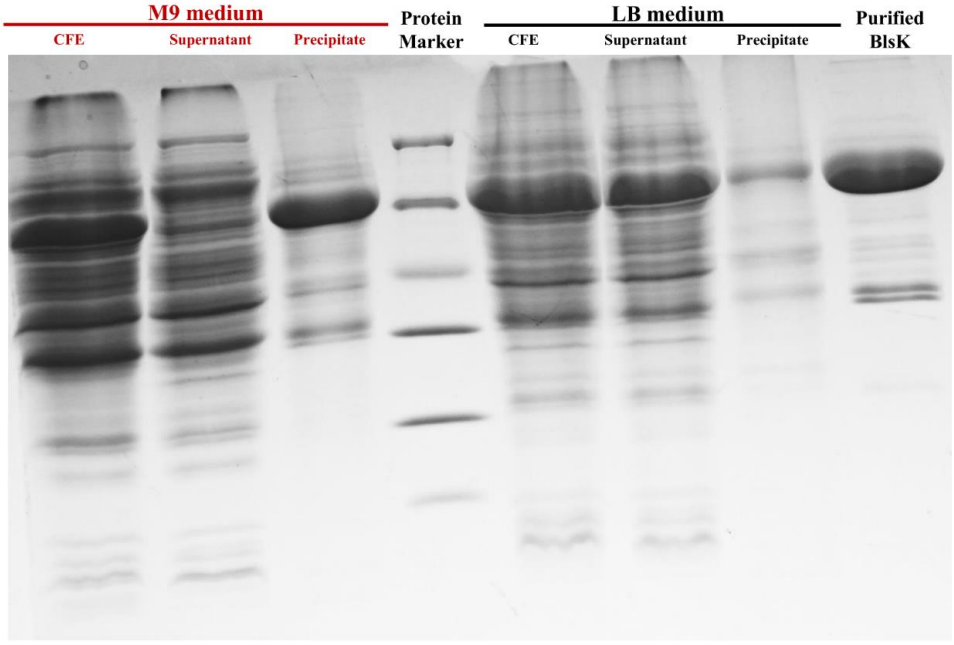

Figure S12. The [3Fe-4S] cluster is critical for the correct folding of BlsK proteins. CFE: whole cell lysate; Supernatant: the soluble part; Precipitate: the insoluble part.

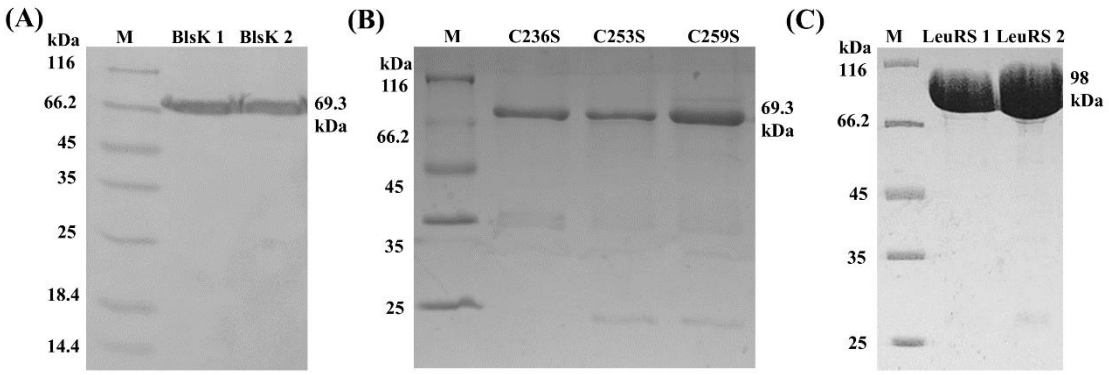

Figure S13. SDS-PAGE analysis of purified proteins used in this study: (A) BlsK1 and BlsK2 are two purified BlsK samples after the Superdex-200 column; (B) three purified BlsK mutants; (C) purified LeuRS with different concentrations.

Reference

1. Li, L., Wu, J., Deng, Z., Zabriskie, T. M. & He, X. (2013) *Streptomyces lividans* blasticidin S deaminase and its application in engineering a blasticidin S-producing strain for ease of genetic manipulation. *Appl Environ Microbiol* 79, 2349-2357.

2. Kieser, T., Bibb, M. J., Chater, K. F., Butter, M. J. & Hopwood, D. A. (2000) Practical *Streptomyces* genetics. John Innes Foundation, United Kingdom, Norwich.

3. Gust, B., Challis, G. L., Fowler, K., Kieser, T. & Chater, K. F. (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci U S A* 100: 1541-1546,

4. Wilkinson, C. J., Hughes-Thomas Z. A., Martin C. J., Böhm I., Mironenko T., Deacon M., Wheatcroft M., Wirtz G., Staunton J., Leadlay P. F. (2002) Increasing the efficiency of heterologous promoters in actinomycetes. *J Mol Microbiol Biotechnol* 4: 417-426 .