

Direct modulation of T-box riboswitch-controlled transcription by protein synthesis inhibitors

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Supplementary Table S1 Sequences of tRNAs and the T-boxes used for the phylogenetic analyses

| org | Acc-stem | D-stem | D-loop | D-stem | Ac-stem | Ac-loop | Ac-stem | V-region | T-stem | T-loop | T-stem | Acc-stem | CCA | | | |
|--------|----------|---------|---------------------------|--------|----------|---------|---------|----------|--------|----------|--------|----------|-------|----|----|----|
| | 1 | 8 | 10 | 14 | 22 | 26 | 27 | 32 | 39 | 44 | 49 | 54 | 61 | 66 | 73 | 74 |
| H. aur | GCGGGAA | TA GCTC | AGT ^T -GGT--A | GAGC | G TCTCC | TTGCCAA | GGAGA | AG---GTC | GCGAG | TTCGAGT | CTCGT | TTCCCGC | T CCA | | | |
| H. aur | GCGGGAG | TA GCGC | AAT--GGT--A | GCGC | A TCTGC | CTGCCAA | GCAGA | TG---GTT | GCGAG | TTCGAGT | CTCGT | CTCCCGC | T CCA | | | |
| H. aur | GCGGGAA | TG GCGC | AAT ^T -GGC--A | GCGC | A TCTGT | TTGCCAA | ACAGA | AG---GTT | GCGGG | TTCGAAC | CCCGT | TTCCCGC | T CCA | | | |
| D. geo | GCGGGAG | TA GCTC | AGC ^T -GGT--A | GAGC | A CTACC | TTGCCAA | GGTAG | AT---GTC | GCGAG | TTCGAAT | CTCGT | CTCCCGC | T CCA | | | |
| D. geo | GCGGGAA | TA GCTC | AGT ^T -GGT--A | GAGC | G TCAGC | TTGCCAA | GCTGA | AT---GTC | GCGAG | TTCGAGT | CTCGT | TTCCCGC | T CCA | | | |
| D. geo | GCGGGAT | TG GTGT | AGT--GGT--A | GCAC | A GCAGC | CTGCCAA | GCTTC | TG---G-C | CTCGG | TTCGAAT | CCGTG | ATCCCGC | T CCA | | | |
| B. sub | GCGGAAG | TA GTTC | AGT--GGT--A | GAAC | A CCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CTTCCGC | T CCA | | | |
| B. sub | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | C TCAGC | CTGCCAA | GCTGA | TG---T-C | GTGAG | TTCGATT | CTCAT | CACCCGC | T CCA | | | |
| B. sub | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | C TCAGC | CTGCCAA | GCTGA | TG---T-C | GTGGG | TTCGATT | CCCAT | CACCCGC | T CCA | | | |
| B. cer | GCGGAAG | TA GTTC | AGT--GGT--A | GAAT | A CAACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CTTCCGC | T CCA | | | |
| B. cer | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | A AGAGC | CTGCCAA | GCTCT | GG---T-C | GAGAG | TTCGATT | CTCTT | CACCCGC | T CCA | | | |
| C. tet | GCGGGAG | TG GCTC | AGT--GGT--A | GAGC | G TCACC | TTGCCAA | GGTGA | AC---GTC | GTGGG | TTCGAAT | CCCAT | CTTCCGC | T CCA | | | |
| C. tet | GCGGGTG | TA GCTC | AAT--GGT--A | GAGT | T CCAGC | CTGCCAA | GCTGG | CT---G-T | GAGAG | TTCGATT | CTCTT | CACCCGC | T CCA | | | |
| C. bot | GCGGGTG | TA ACTC | AGT--GGT--A | GAGT | G CTAGC | CTGCCAA | GCTAG | TT---A-C | GAGGG | TTCGAAT | CCCGT | CTTCCGC | T CCA | | | |
| C. bot | GCGGAG | TA GTTC | AGT--GGT--A | GAAC | A CTAGC | TTGCCAA | GCTAG | TT---GCC | GCGGG | TTCGATC | CCCGT | TTCTCGC | T CCA | | | |
| C. bot | GCGGGAG | TG GCTC | AGT--GGT--A | GAGC | G TCACC | TTGCCAA | GGTGA | AC---GTC | GCGAG | TTCGAAT | CTCGT | CTTCCGC | T CCA | | | |
| C. ace | GCGGGAA | TA GCTC | AGT--GGT--A | GAGC | A CTAGC | TTGCCAA | GCTGG | GT---GCC | GCGGG | TTCGATA | CCCGT | TTCCCGC | T CCA | | | |
| C. ace | GCGGGAG | TG GCTC | AGT--GGT--A | GAGC | G TCACC | TTGCCAA | GGTGA | AC---GTC | GCGAG | TTCGAAT | CCCGT | CTTCCGC | T CCA | | | |
| C. ace | GCGGGTG | TA ACTC | AAT--GGT--A | GAGT | G CTAGC | CTGCCAA | GCTAG | TT---A-C | GAGGG | TTCGATT | CCCTC | TACCCGC | T CCA | | | |
| C. dif | GCGGGAA | TA GTTC | AGT--GGT--A | GAGC | G CAACC | TTGCCAA | GGTGG | AA---GTC | GCGAG | TTCGAAT | CTCGT | TTCCCGC | T CCA | | | |
| C. dif | GCGGGTG | TA GCTC | AAT--GGT--A | GAGT | T CTGGC | CTGCCAA | GCCAG | CT---G-T | GAGGG | TTCGATC | CCCTT | CACCCGC | T CCA | | | |
| E. fae | GCGGAAA | TA GCTC | AGT--GGT--A | GAGC | A CCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | TTTCCGC | T CCA | | | |
| E. fae | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | C ACAGC | CTGCCAA | GCTGT | TG---T-C | GCGAG | TTCGATT | CTCGT | CACCCGC | T CCA | | | |
| S. aur | GCAGAAG | TA GTTC | AGC--GGT--A | GAAT | A CAACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CTTCTGC | T CCA | | | |
| S. aur | GCGGGTG | TA GTTT | AAT--GGC--A | AAAC | C TCAGC | CTGCCAA | GCTGA | TG---T-T | GTGGG | TTCGATT | CCCAT | CACCCGC | T CCA | | | |
| S. aur | GCGGGAG | TA GTTC | AACT ^T -TTT--A | GAAC | A CGTTC | CTCCCG | GAACG | AG---G-T | ATGGG | TGCAAAAT | CCATAT | CTTCCGC | T CCA | | | |
| S. aur | GCGGGAG | TA GTTC | AACT ^T -TTT--A | GAAC | A CGTTC | CTCCCG | GAACG | AG---G-T | ATGGG | TGTAAAT | CCATAT | CTTCCGC | T CCA | | | |
| S. aur | GCGGGAG | TA TTTC | AACT ^T -CTT--A | GAAT | A CATTTC | CTCCCTG | GAATG | AG---G-T | ATGGG | TGTAAAT | CCATAT | CTTCCGC | T CCA | | | |
| S. epi | GCGGAAG | TA GTTC | AGT--GGT--A | GAAC | A CCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CTTCCGC | T CCA | | | |
| S. epi | GCGGGTG | TA GTTT | AAT--GGC--A | AAAC | C TCAGC | CTGCCAA | GCTGA | TG---T-T | GTGGG | TTCGATT | CCCAT | CACCCGC | T CCA | | | |
| S. epi | GCGGGAG | TA GTTC | AACT ^T -CTC--A | GAAC | A CATTTC | CTCCCG | GAATG | AG---A-T | ATGGG | TGTAAAT | CCATAT | CTTCCGC | T CCA | | | |
| S. epi | GCGGGAG | TA GTTC | AACT ^T -CTC--A | GAAC | A CATTTC | CTCCCG | GAATG | AG---A-T | ATGGG | TGTAAAT | CCATAT | CTTCCGC | T CCA | | | |
| S. epi | GCGGGAG | TA GTTC | AACT ^T -CTT--A | GAAC | A CATTTC | CTCCCG | GAATG | AG---A-T | ATGGG | TGTAAAT | CCATAT | CTTCCGC | T CCA | | | |
| S. sap | GCAGAAG | TA GTTC | AGC--GGT--A | GAAT | A CGACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CTTCTGC | T CCA | | | |
| S. sap | GCGGGTG | TA GTTT | AAT--GGC--A | AAAC | C TCAGC | CTGCCAA | GCTGA | TG---T-T | GTGGG | TTCGATT | CCCAT | CACCCGC | T CCA | | | |
| S. san | GCGAACG | TA GTTC | AGT--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CGTTCGC | T CCA | | | |
| S. san | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | T ACAGC | CTGCCAA | GCTGT | TG---T-C | GCGAG | TTCGATT | CTCGT | CACCCGC | T CCA | | | |
| S. mut | GCGAACG | TA GTTC | AGT--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CGTTCGC | T CCA | | | |
| S. mut | GCGAACG | TA GTTC | AGT--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCAT | CGTAGGT | T CCA | | | |
| S. mut | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | C ACAGC | CTGCCAA | GCTGT | TG---T-C | GCGAG | TTCGATT | CTCGT | CACCCGC | T CCA | | | |
| S. mut | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | T ACAGC | CTGCCAA | GCTGT | TG---T-C | GCGAG | TTCGATT | CTCGT | CACCCGC | T CCA | | | |
| S. pne | GCGAACG | TA GTTC | AGT--GGT--A | GAAC | A CCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CGTTCGC | T CCA | | | |
| S. pne | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | T ACAGC | CTGCCAA | GCTGT | TG---T-C | GCGAG | TTCGATT | CTCGT | CACCCGC | T CCA | | | |
| S. aga | GCGAACG | TA GTTC | AGT--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGA | GG---GTC | GCGGG | TTCGAAC | CCCGT | CGTTCGC | T CCA | | | |
| S. aga | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | T ACAGC | CTGCCAA | GCTGT | TG---T-C | GCGAG | TTCGATT | CTCGT | CACCCGC | T CCA | | | |
| L. inn | GCGGAAG | TA GTTC | AGT--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAC | CCCGT | CTTCCGC | T CCA | | | |
| L. inn | GCGGAAG | TA GTTC | AGC--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAT | CCCGT | CTTCCGC | T CCA | | | |
| L. inn | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | T ACAGC | CTGCCAA | GCTGT | TG---T-C | GTGGG | TTCGATT | CCCAT | CACCCGC | T CCA | | | |
| L. mon | GCGGAAG | TA GTTC | AGT--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAC | CCCGT | CTTCCGC | T CCA | | | |
| L. mon | GCGGAAG | TA GTTC | AGC--GGT--A | GAAC | A TCACC | TTGCCAA | GGTGG | GG---GTC | GCGGG | TTCGAAC | CCCGT | CTTCCGC | T CCA | | | |
| L. mon | GCGGGTG | TA GTTT | AGT--GGT--A | AAAC | T ACAGC | CTGCCAA | GCTGT | TG---T-C | GTGGG | TTCGATT | CCCAT | CACCCGC | T CCA | | | |

H. aur GCGAATGGCGACGGGGAGCCCAATCACAATAGATCTCAAAGTGCCTTGGTTCAACCAAGGAACTGGGGTGAACCCGG
D. geo GCGAAGGGCGGCGGGGAGCTAATCCTTCTGCAAAAGTCCGGGGTGGACGGACTGTTCTCCCGAACTGGGGTGAACCCGG
B. sub ACGAAGGGCATTCTGAGCAATTTAAAAAGAGGCTGGGATTTGTCTCAGCACTAGGGTGAACCCGG
B. cer GGAAGGGCAGTCTCGAGCAATACGTGATAAAGTGGATGCACCTTTTGTGTATCAACTAGGGTGAACCCGG
C. tet GTGAATGGAAGCTCAAGAGTGTAAATGTAGAGCAGGGCTGTTGCCAAAAGGGTGAACCCGG
C. bot ATGAAGGGAAGCTTTTGTAGTATTTAATTAGAAAGTAGGGCTATAGCCA AAAAGGGTGAACCCGG
C. ace AGTAGTGAAGTCTTTCGAGTATTTTTAAAAAGAAAAGCAGGGCTATTGCCAATAAGGGTGAACCCGG
E. fae TTGAAGGGCCTTTTGGAGTACGACAAACGAAGCTGCCGATGAACACATCGGAAGTGGGTGAACCCGG
S. aur AAGATTGGCCCAACGAATCATTTTTAAAAATAAAGCGAGTGACTACACTAATTTGGGTGAACCCGG
S. epi TATTTTGGCATTATGATTAATTTTTATAAAGCGAGTGTTTACACTAATTTGGGTGAACCCGG
S. sap AACCAATGGCTAAAATTAATGATAGTTAAAAGCGAGTGTACACACTAATTTGGGTGAACCCGG
S. san GCAGATGGCCTTTCGGTTATAATTTCAAAGTAATGAAGTAATAAATTAGGGTGAACCCGG
S. mut AGGACTGGCACTTTCTCTTGGCTAATAGCCAAGCTAACCAATCAGATAAATGAAGTAATAAATTAGGGTGAACCCGG
S. pne AGGGCTGGCCTTTCTGTAGTATTTTTAAAAACAATGAAGTAATAAATTAGGGTGAACCCGG
S. aga AATATTGGCACTTTTGTAGTAAAAGTACAATCAAATGAAGTAATAAATTAGGGTGAACCCGG
L. inn TGTGAAGGGCTTCTGGAGTACAGCGAAATCAAGTGGGAATGTTTAAATTTCCAAATAGGGTGAACCCGG
L. mon TGTGAAGGGCTTCTGGAGTACAGCGAAATCAAGTGGGAATGTTTAAATTTCCAAATAGGGTGAACCCGG

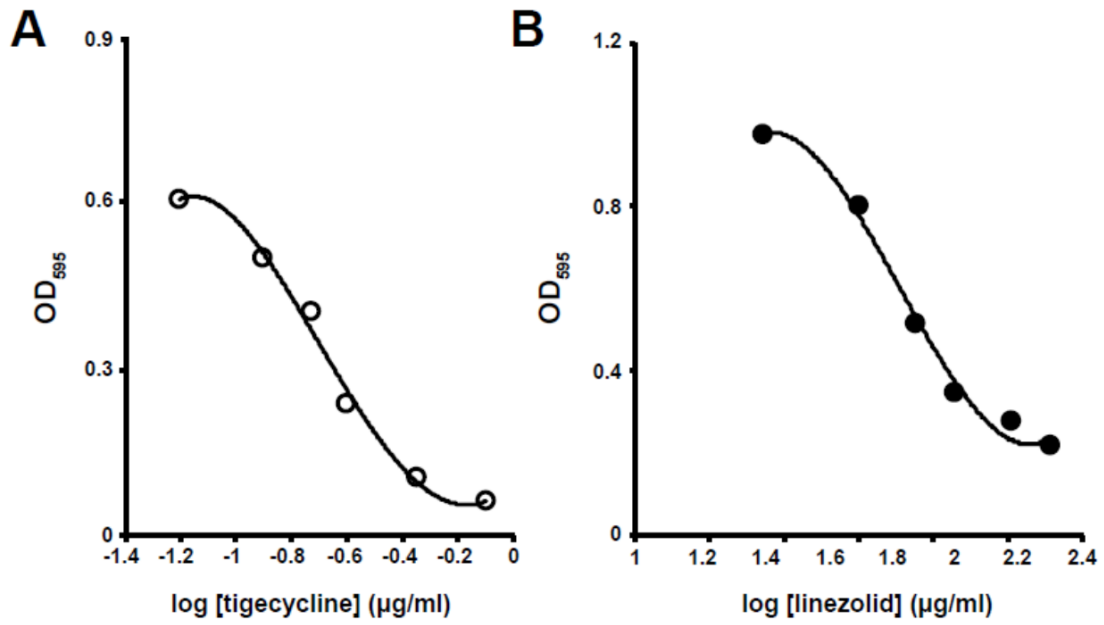
The annotation numbers used are: Haur_4697: *H. aurantiacus* ATCC 23779; Dgeo_1716: *D. geothermalis* DSM 11300; BSU25270: *B. subtilis* str. 168; BCE_5053: *B. cereus* ATCC 14579; CTC00212: *C. tetani* E88; CBO3517: *C. botulinum* str. ATCC 3502; CAC3195: *C. acetobutylicum* ATCC 824; EF2408: *E. faecalis* V583; SA1394: *S. aureus* N315; SE1252: *S. epidermidis* ATCC 12228; SSP1191: *S. saprophyticus* ATCC 15305; SSA_1881: *S. sanguinis* SK36; SMU_444: *S. mutans* UA159; SP_1477: *S. pneumoniae* TIGR4; SAG0268: *S. agalactiae* 2603V/R; lin1496: *L. innocua* Clip11262; lmo1459: *L. monocytogenes* EGD-e.

SUPPLEMENTARY FIGURES LEGENDS

Supplementary Figure S1 3D representation of tRNA^{Gly}_{GCC}. The main positions that were found protected by tigecycline and neomycin B are indicated.



Supplementary Figure S2 IC₅₀ values calculation for tigecycline and linezolid (0.103 µg/ml and 85.43 µg/ml, respectively).



Supplementary Methods

In vitro transcription and purification of *S. aureus* GT-box and P1 tRNA^{Gly}_{GCC}

The *S. aureus* GT-box and P1 tRNA^{Gly}_{GCC} are cloned into pUC57 vector by GenScript and pUC18 vector, respectively (33). Both plasmid constructs were designed with a T7 promoter leader sequence and the terminal BstNI restriction enzyme recognition site. After BstNI digestion and linearization, the plasmids were treated with phenol:chloroform:isoamyl alcohol (25:24:1), precipitated with ethanol and used as template for subsequent *in vitro* transcription using T7 RNA polymerase. The linearization efficiency was confirmed on a 1% agarose gel. Run-off *in vitro* transcription reactions were carried out at 30°C for the GT-box and 37°C, for the P1 tRNA^{Gly}_{GCC} for 16 h. The reactions mixtures contained 10 µg of purified linear plasmids as template, 500U T7 RNA polymerase (Takara), 2 mM of each ribonucleotide (rUTP, rGTP, rCTP, rATP), 160 U RNasin (Takara), 8U inorganic pyrophosphatase (New England Biolabs) and DTT (5mM final concentration) up to a final volume 500µL. The reactions were stopped by placing the mixture on ice followed by DNase I digestion of the template, phenol:chloroform:isoamyl alcohol (25:24:1) extraction, and ethanol precipitation. Subsequently the transcripts were purified on an 8 or 10% PAGE/8M urea. The band corresponding to the correct transcript length was excised after visualization under a UV lamp and the transcript was eluted in the presence of buffer containing 10mM Tris-HCl pH7.5, 300mM KCl, 1mM EDTA at 4°C for 16 h in continuous shaking. The eluted transcripts were ethanol precipitated, denatured by heating at 65 °C for 5 min and refolded with slow cooling in the presence of the same buffer with the addition of 1 mM MgCl₂. After refolding the transcripts were loaded on a gel filtration column (Superdex 200 10/ 300 GL-ÄKTA FPLC system) using the same buffer to be further purified from nucleotides and unwanted bulky conformations (i.e. dimers), unless indicated otherwise. After elution, the properly folded and structured transcripts were analysed for size and quality on an 8 or 10% PAGE/8M Urea. Representative chromatographs and gels stained with methylene blue (0.025%) are presented in Supplementary Figure S3.

Supplementary Figure S3 Chromatographs corresponding to purification of T-box (upper panel) and P1 tRNA (lower panel) on a gel filtration column column (Superdex 200 10/ 300 GL-ÅKTA FPLC system). The inserts correspond to eluted transcripts analysed for purity and correct size on 8 or 10% PAGE/8M urea.

