

**Supplementary information**

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**Structural basis for context-specific inhibition of translation by oxazolidinone antibiotics**

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Supplementary Information for:

**Structural basis for context-specific inhibition of translation by  
oxazolidinone antibiotics**

**Short title:** Translation inhibition by oxazolidinone antibiotics

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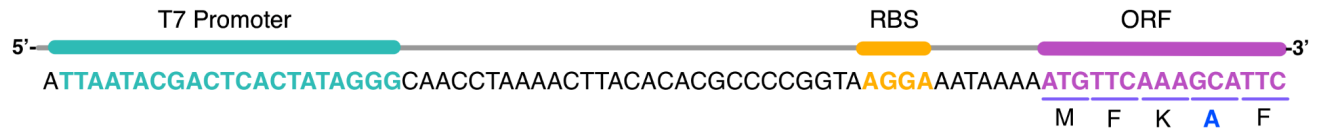
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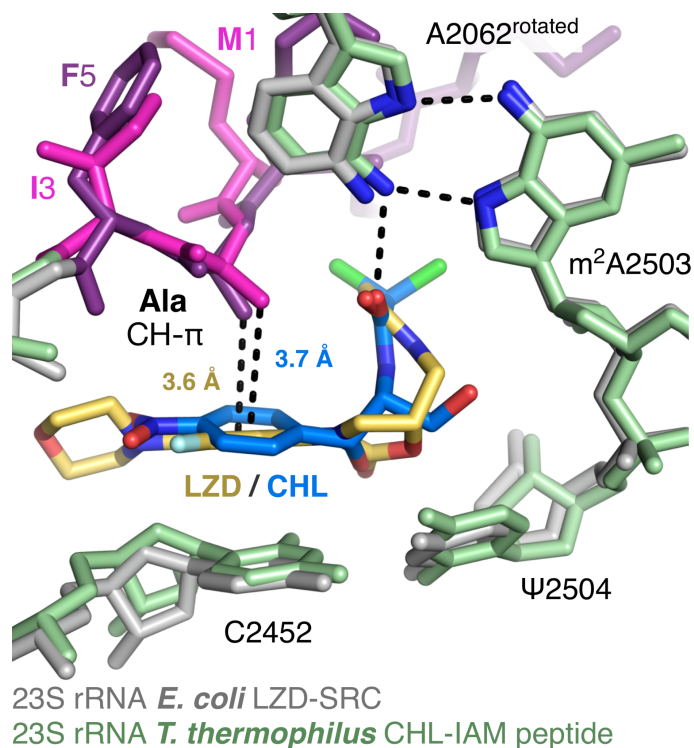
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**DNA template:**

**Supplementary Fig. 1. Preparation of oxazolidinone-stalled ribosome complexes for cryo-EM analysis.** Architecture of the DNA template encoding the T7 promoter, ribosome binding site (RBS), and model MFKAF stalling peptide open reading frame (ORF). Generation of stalled ribosomes at the F5 codon was biased by not including a stop codon at the end of the stalling peptide ORF.



**Supplementary Fig. 2. Linezolid and chloramphenicol use the same interaction with alanine for context-specificity.** Overlay was performed by alignment of 23S rRNA nucleotides 2000-3000, highlighting the CH- $\pi$  interaction between the aryl ring of linezolid (LZD, yellow) or chloramphenicol (CHL, blue)<sup>1</sup> and the penultimate alanine side chain. Tri-peptide corresponding to the CHL complex is shown in pink, and the nascent chain from LZD-SRC is shown in purple. Labeling of 23S rRNA corresponds to *E. coli* numbering.

Primer name	Sequence
T7	5'-ATTAATACGACTCACTATAGG-3'
ORF_SD	5'-GAATGCTTTGAACATTTTTATTTC-3'
T7_MFKAF_Fwd	5'-ATTAATACGACTCACTATAGGGCAACCTAAACTTACACACGCCCCG-3'
SD_MFKAF_Rev	5'-GAATGCTTTGAACATTTTTATTTCCTACCGGGCGTGTGTAAGTTTTAG-3'

**Supplementary Table 1. Primer sequences used in this study.** Oligonucleotides were purchased from Integrated DNA Technologies and prepared with standard desalting procedures.

**SUPPLEMENTARY INFORMATION REFERENCES**

1. Syroegin, E. A. *et al.* Structural basis for the context-specific action of classic peptidyl transferase inhibitors. *bioRxiv* 2021.06.17.448903 (2021) doi:10.1101/2021.06.17.448903.