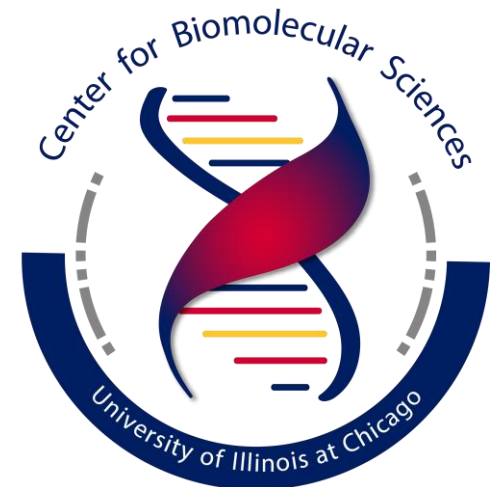
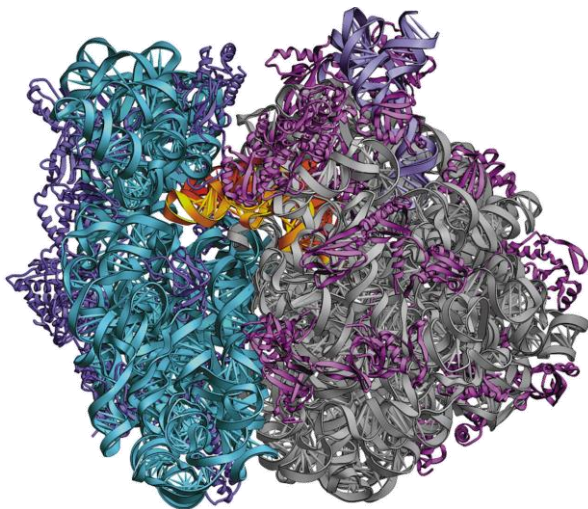
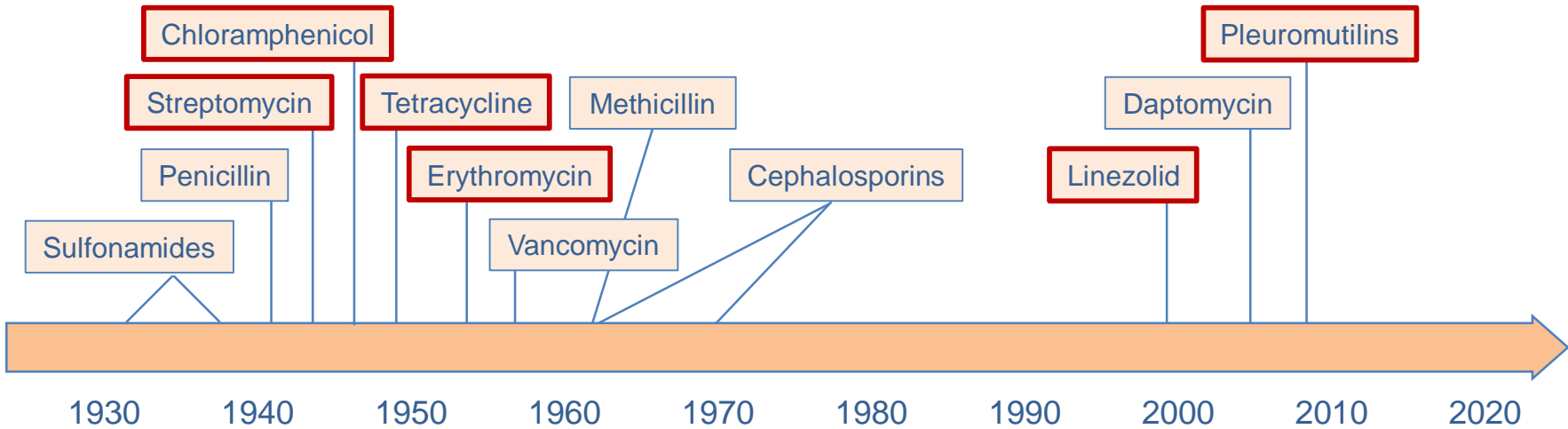


Modulating the ribosome function by small molecules

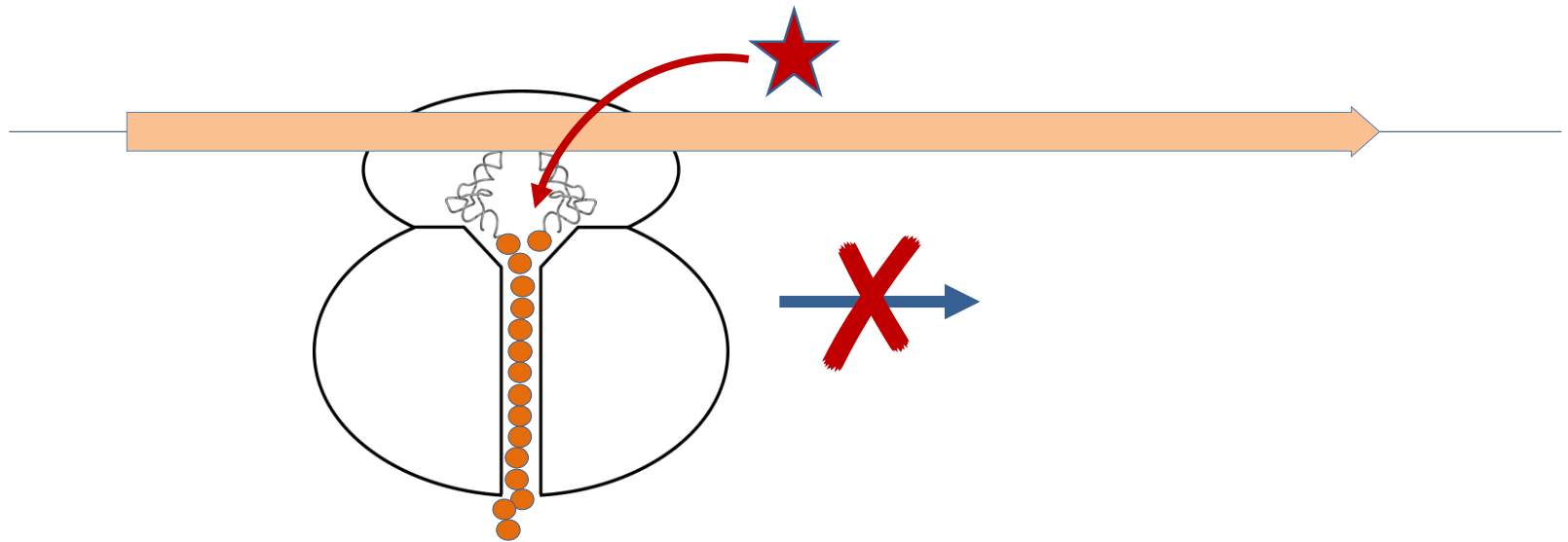
Alexander Mankin

Center for Biomolecular Sciences
University of Illinois at Chicago

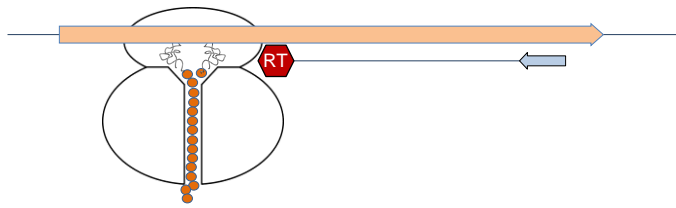




Traditionally, all ribosomal antibiotics were viewed as global inhibitors of translation



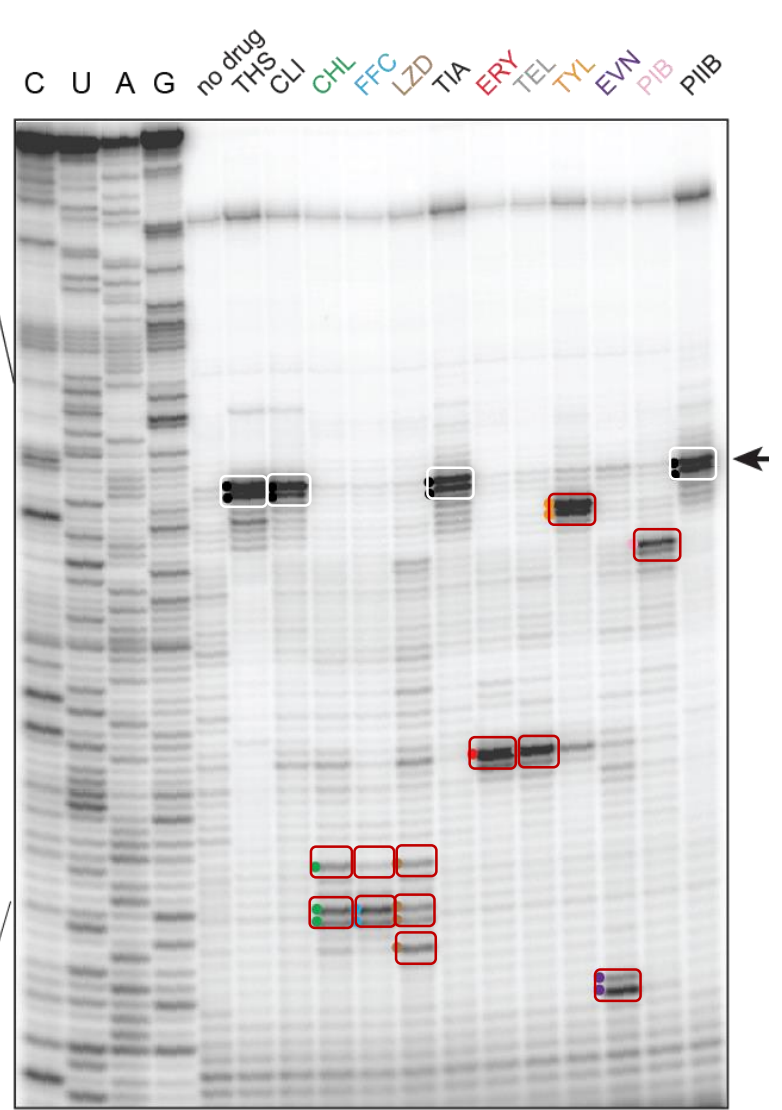
Most antibiotics that target large ribosomal subunit arrest the ribosome at specific mRNA sites



THS: thiostrepton
CLI: clindamycin
CHL: chloramphenicol
FFC: florfenicol
LZD: linezolid
TIA: tiamulin

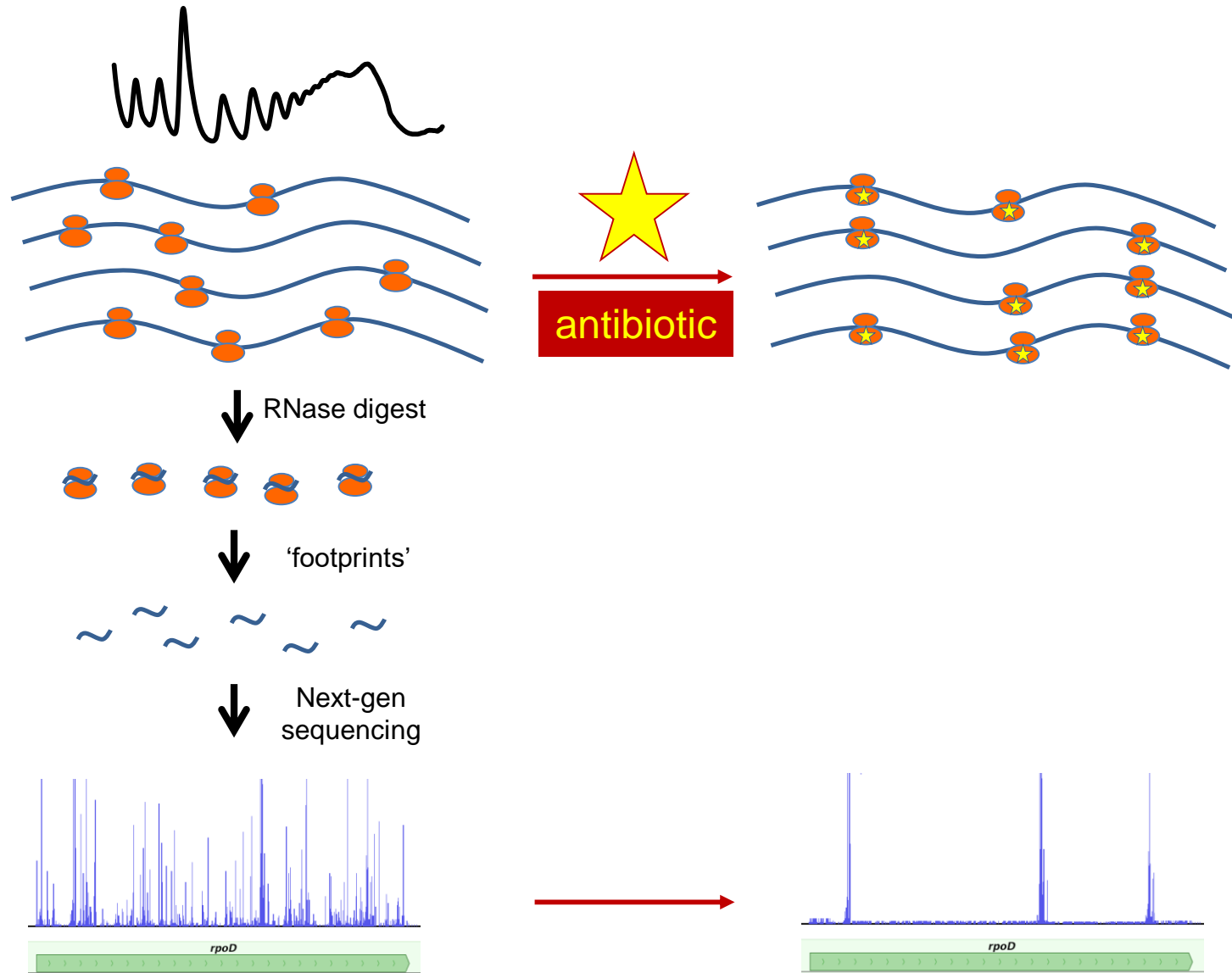
ERY: erythromycin
TEL: telithromycin
TYL: tylosin
EVN: evernimicin
PIB: pristinamycin IB
PIIB: pristinamycin IIB

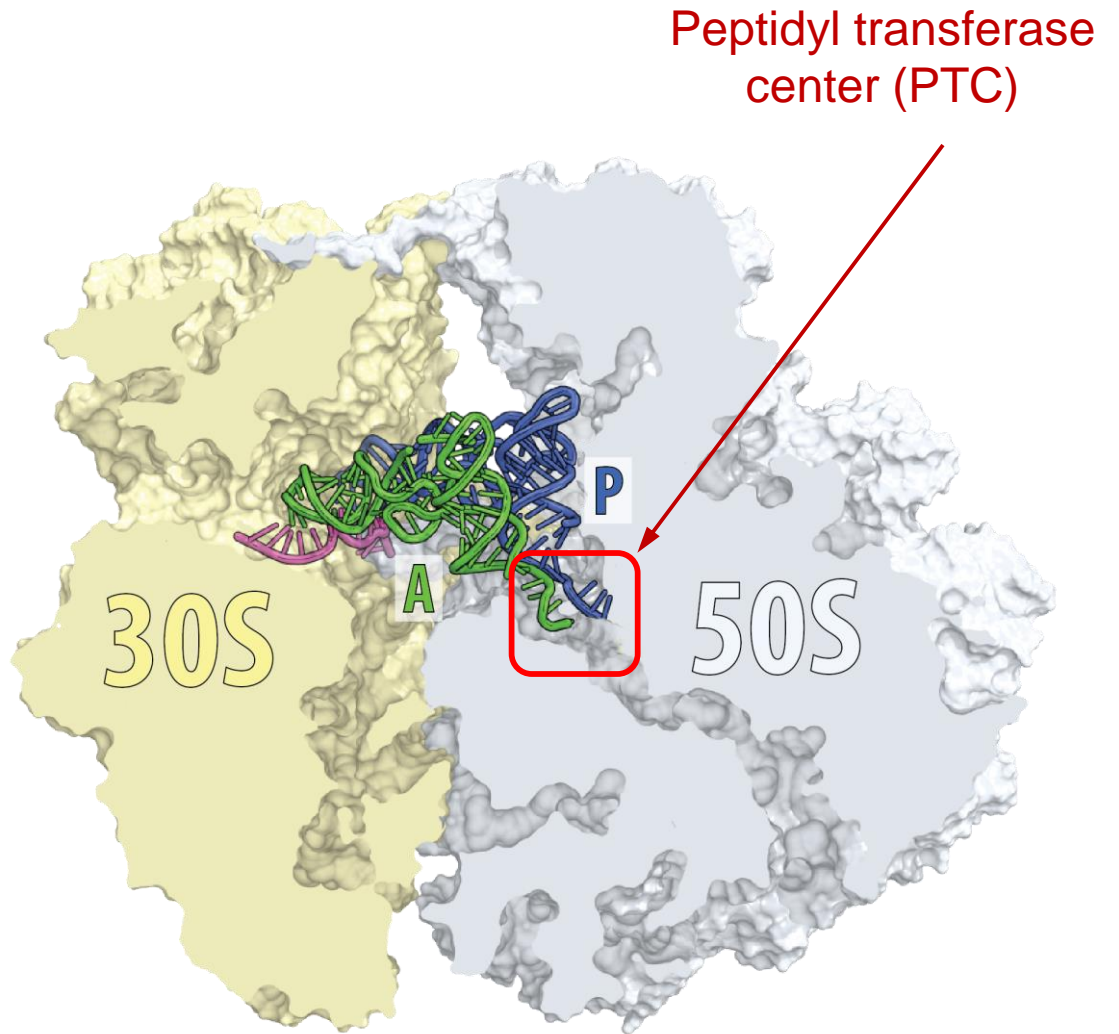
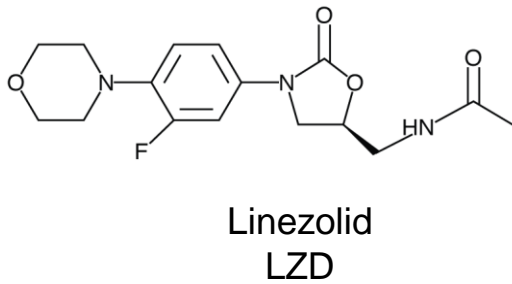
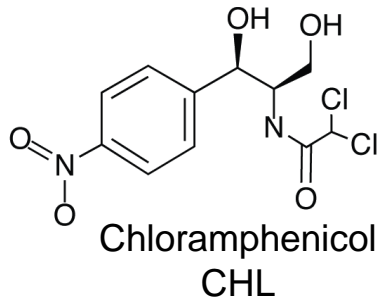
• M A U G U U G G U A U U C C A A A U G C G U A A U G U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • L U U G G U A U U C C A A A U G C G U A A U G U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • F U U C C A A A U G C G U A A U G U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • Q A A A U G C G U A A U G U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • M U G C G U A A U G U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • R G U A A U G U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • N A A U G U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • V U A G A U A A A C A U C U A C U A U U U G A A A U A A
 • D A U A A A C A U C U A C U A U U U G A A A U A A
 • K A A A C A U C U A C U A U U U G A A A U A A
 • T A C A U C U A C U A U U U G A A A U A A
 • S C U A C U A U U U G A A A U A A
 • T C U A C U A U U U G A A A U A A
 • I U U U G A A A U A A
 • L U U U G A A A U A A
 • K A A A U A A
 • * A A A

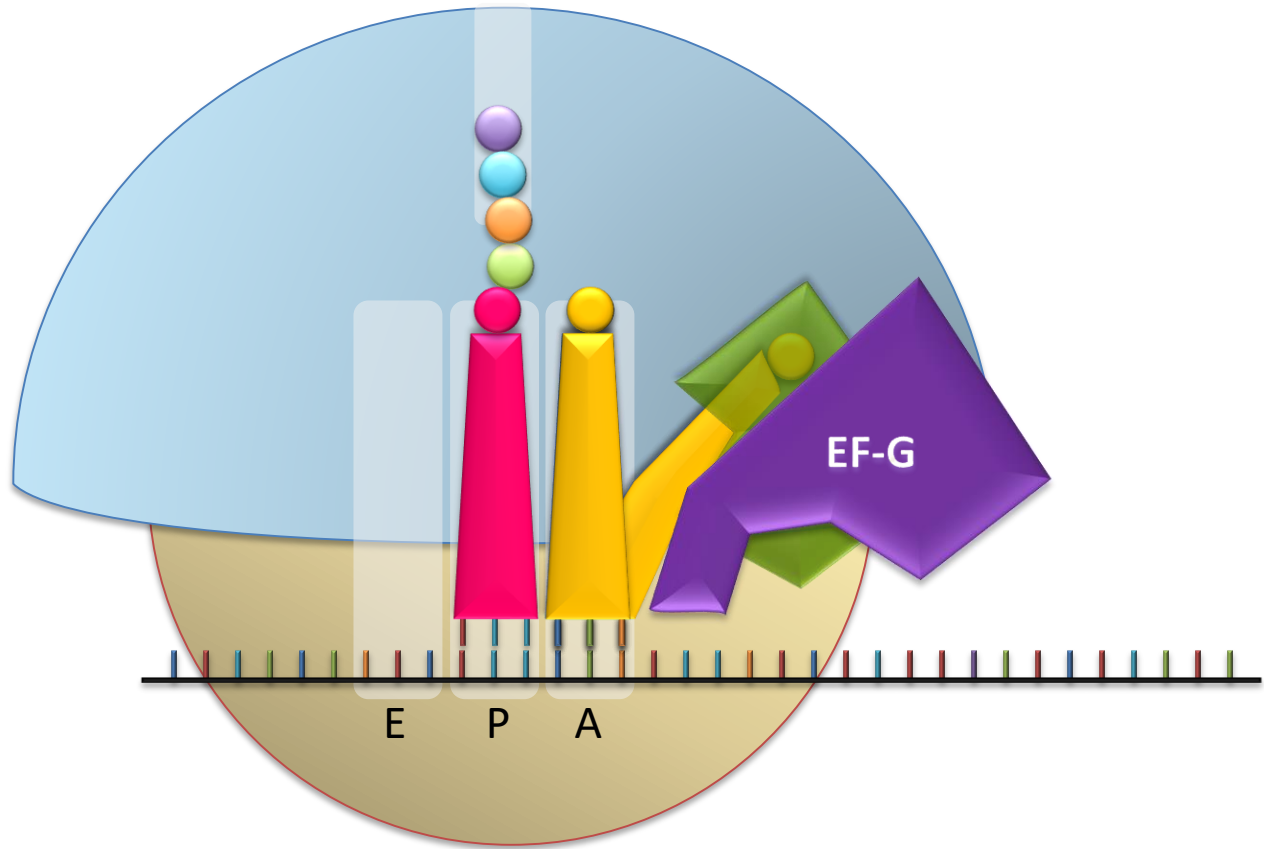


What are the molecular mechanisms of context specificity of antibiotic action?

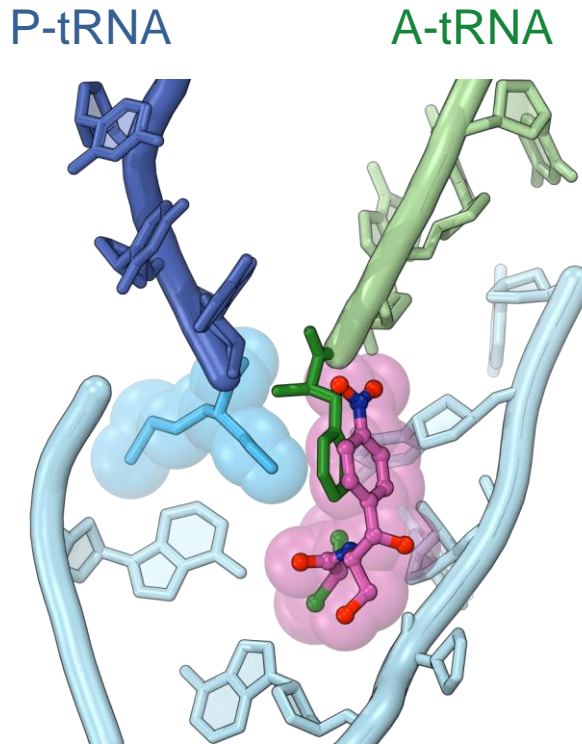
Ribosome profiling as a tool for revealing context-specificity of antibiotic action



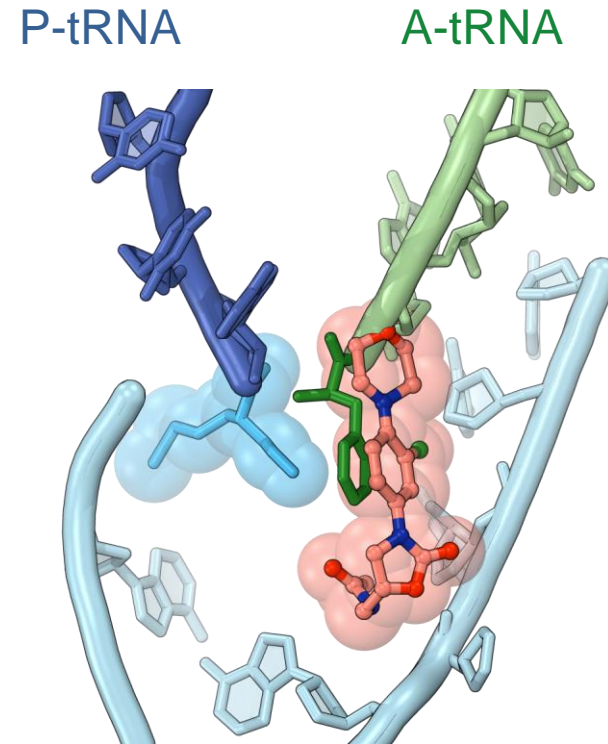




CHL and LZD should inhibit formation of **every** peptide bond because they should compete with **any** aminoacyl-tRNA

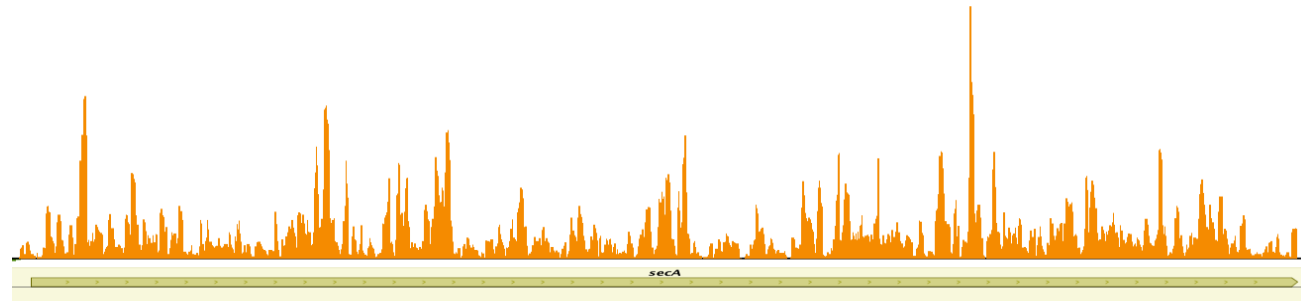


Chloramphenicol

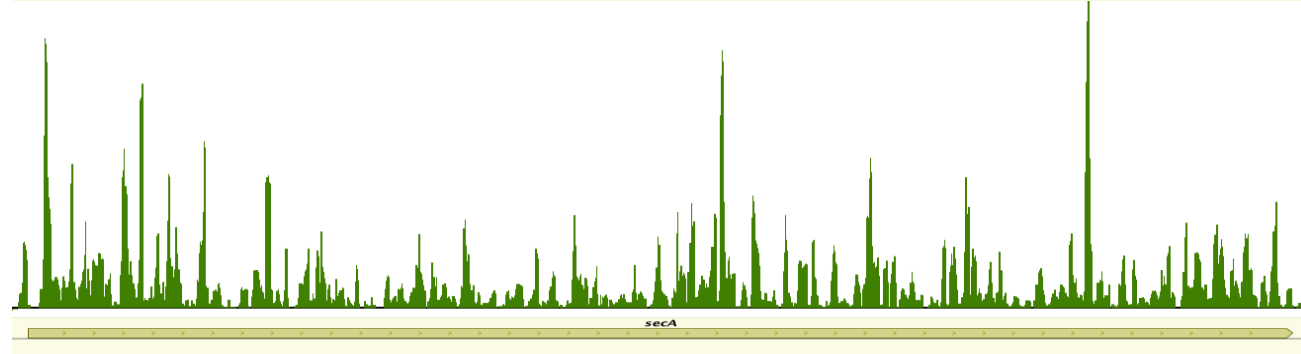


Linezolid

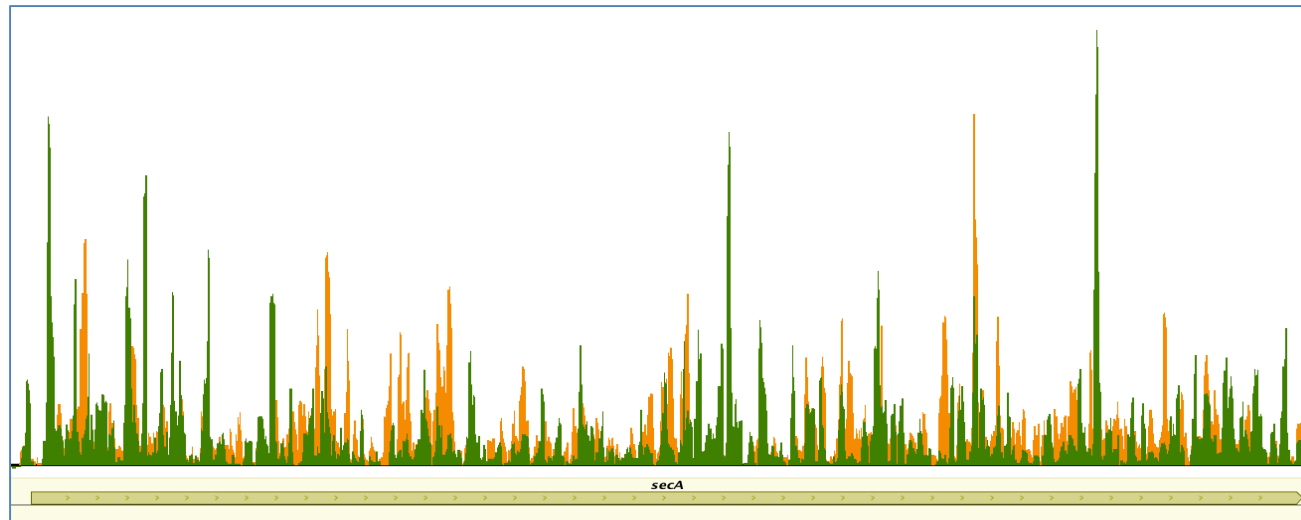
CHL and LZD cause redistribution of ribosomes on mRNAs



no drug

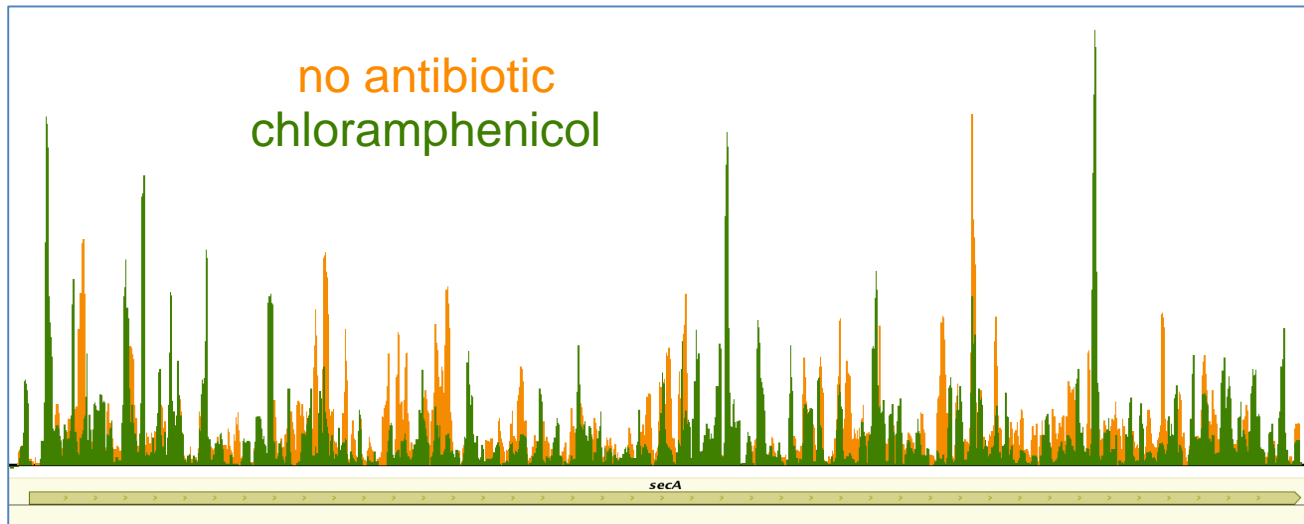


chloramphenicol

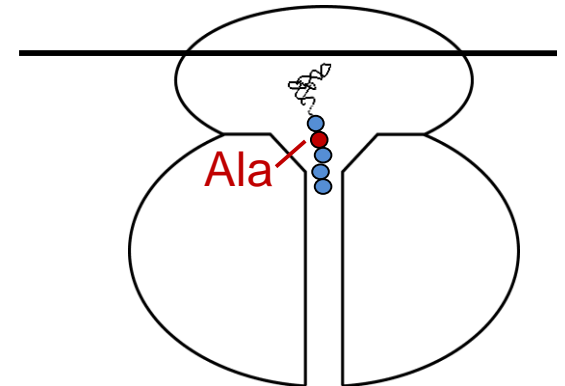
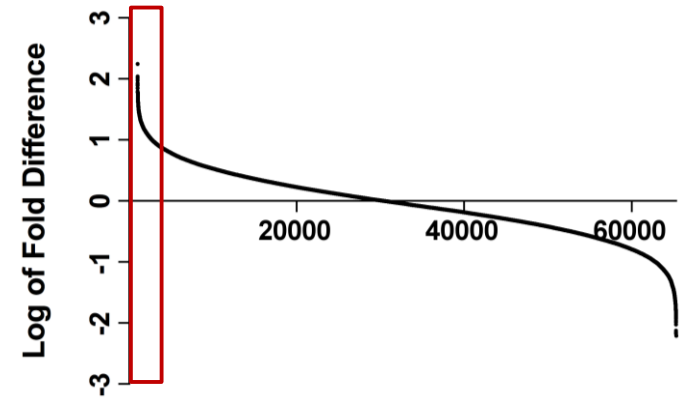
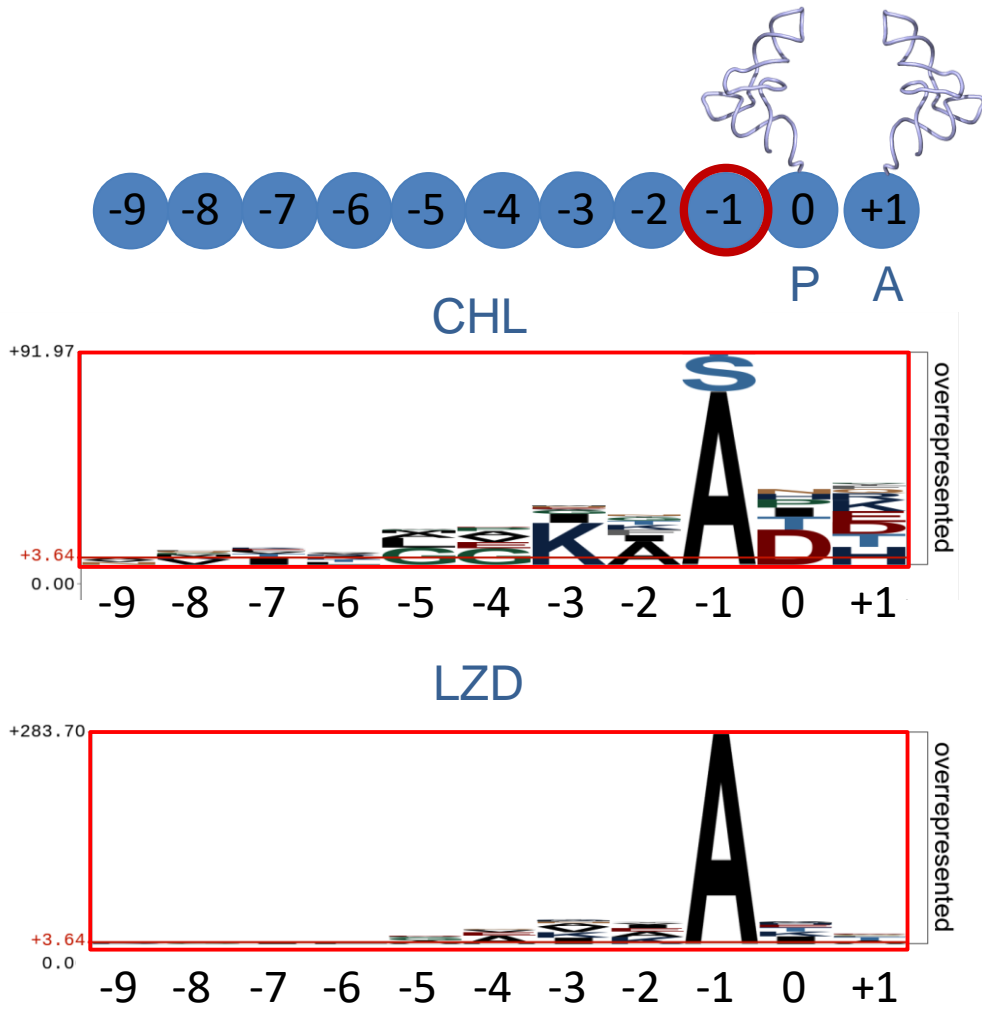


overlay

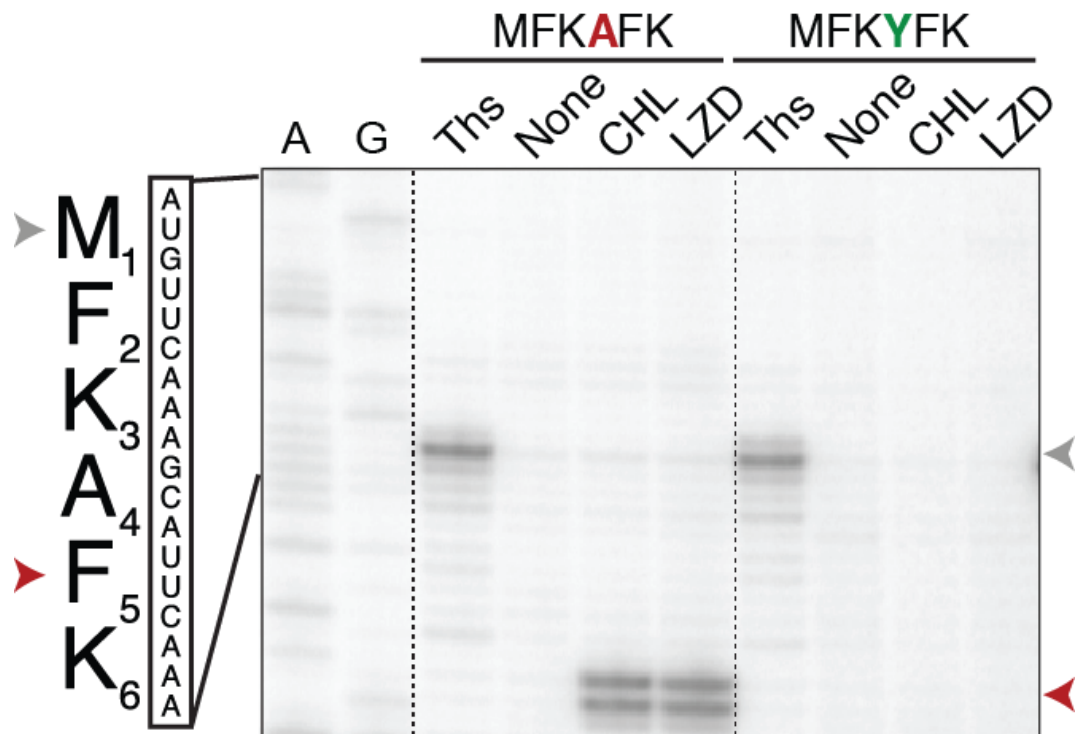
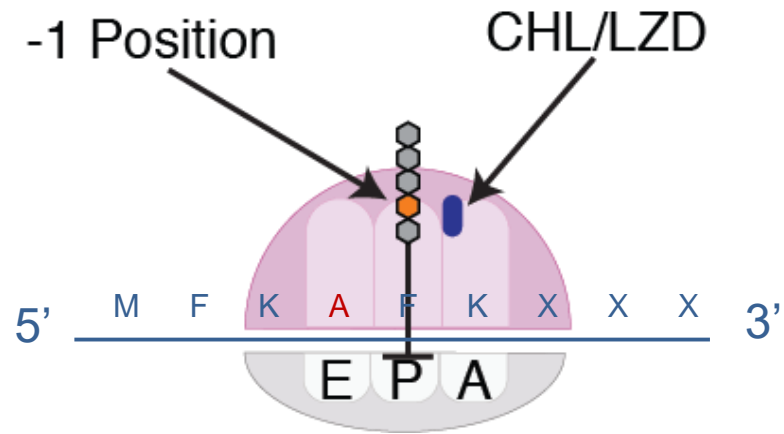
What are the sites where CHL and LZD arrest translation?

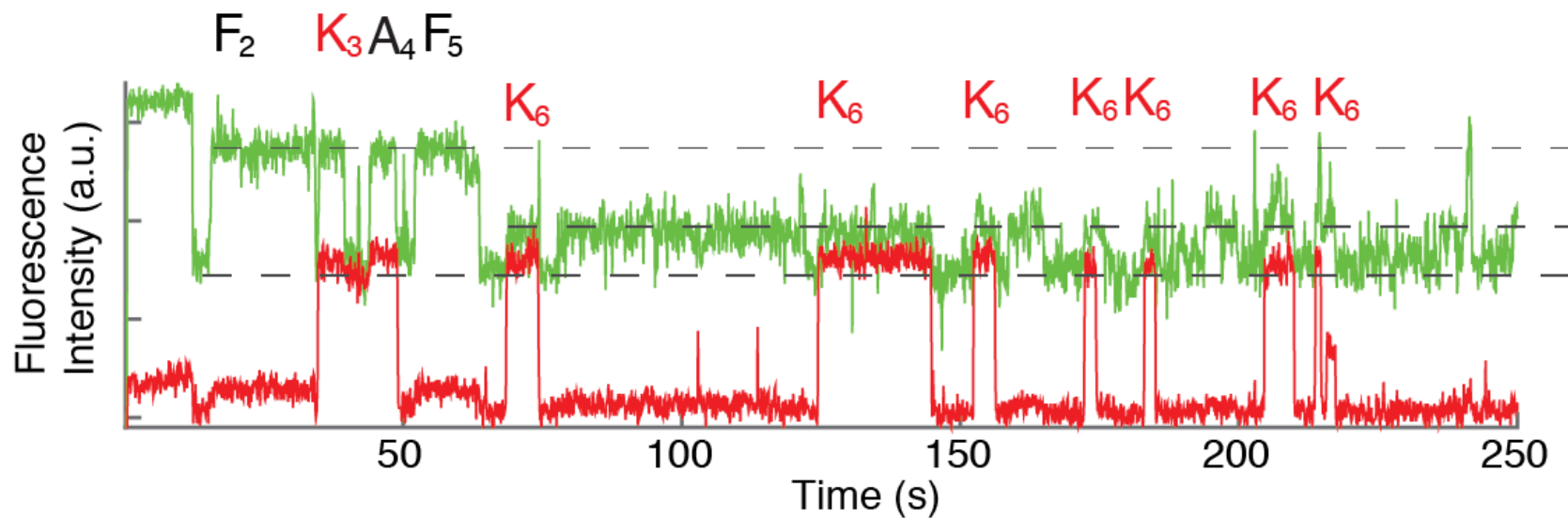
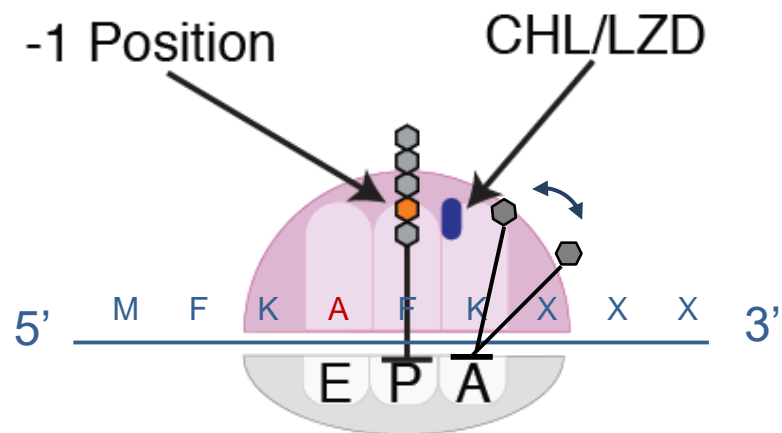


calculate difference ('fold change') in the ribosome density codon-by-codon



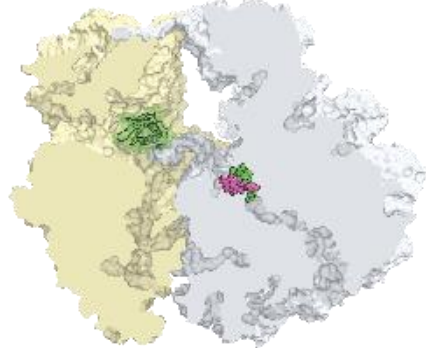
Linezolid and chloramphenicol predominantly inhibit translation when Ala appears in the penultimate position of the nascent protein





The penultimate amino acid of the nascent protein participates in creating a high-affinity antibiotic site

T. thermophilus



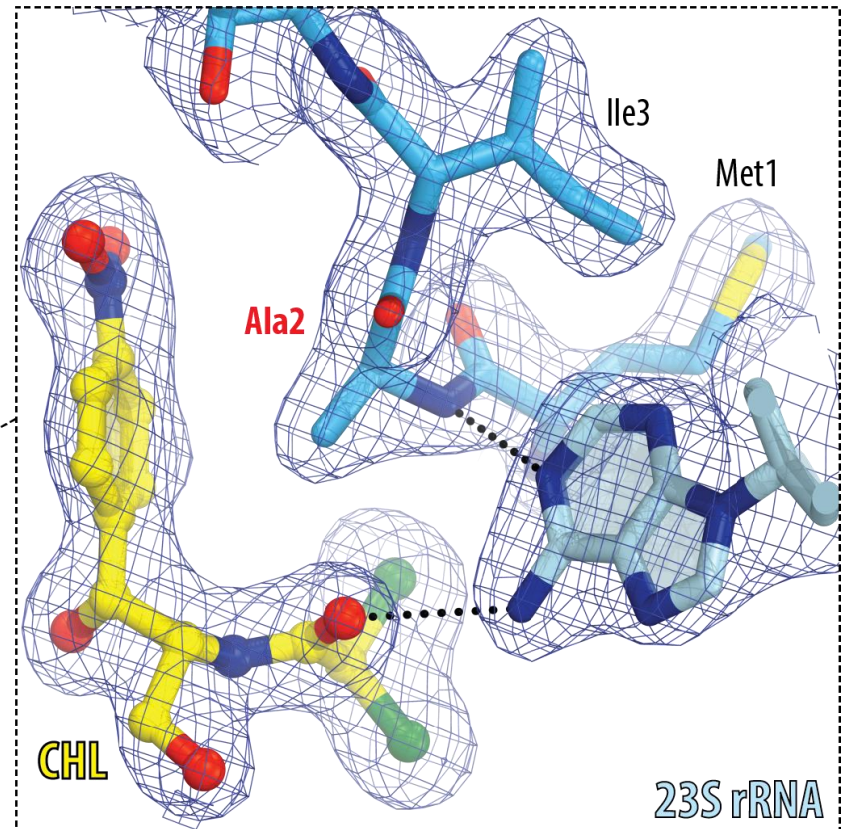
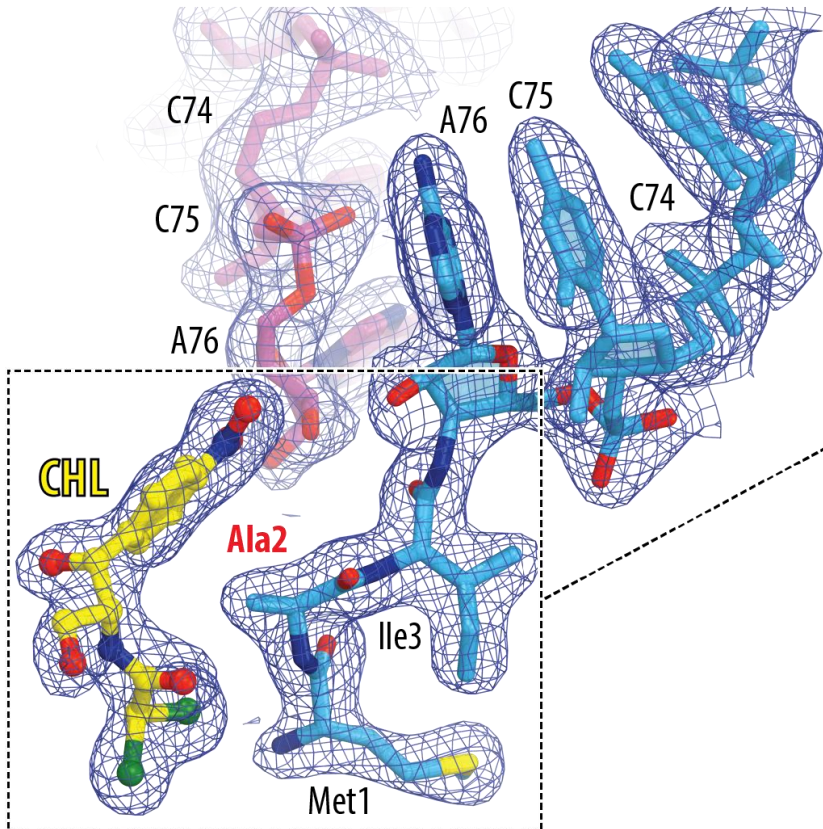
P site: Met-Ala-Ile-ACCA

A site: CACCA

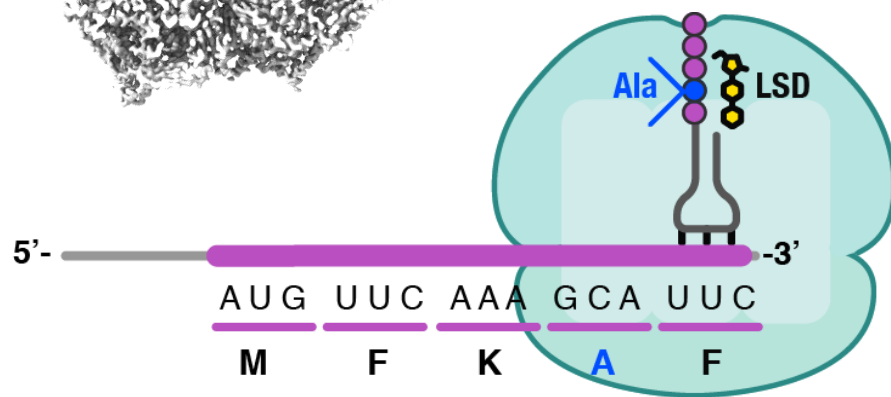
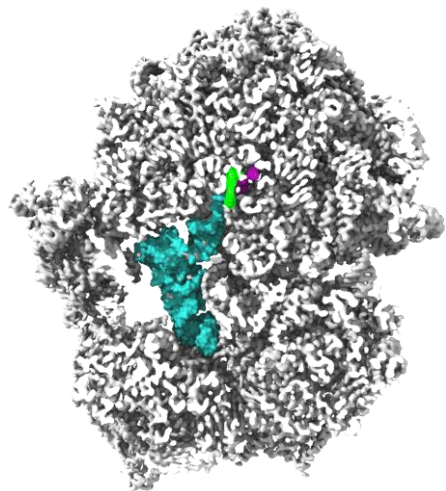
CHL

A-site

P-site

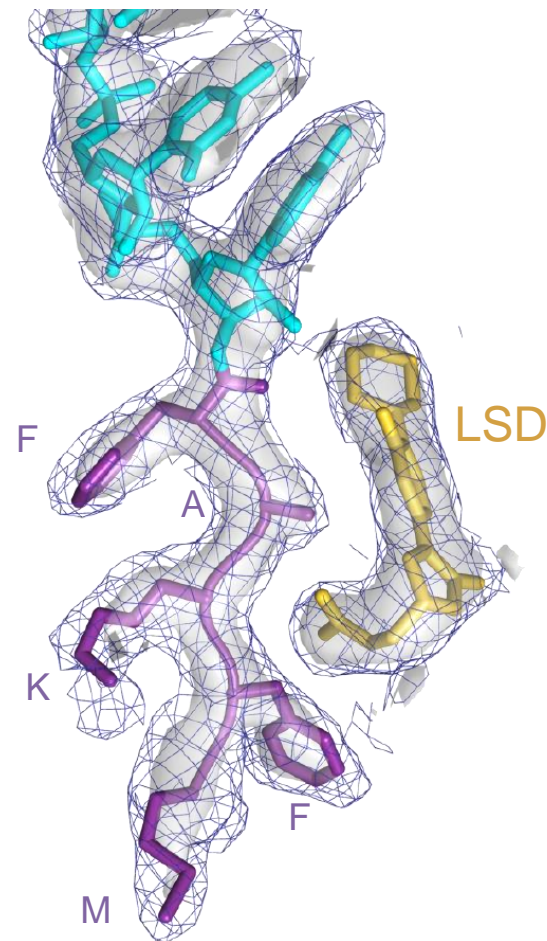


E. coli

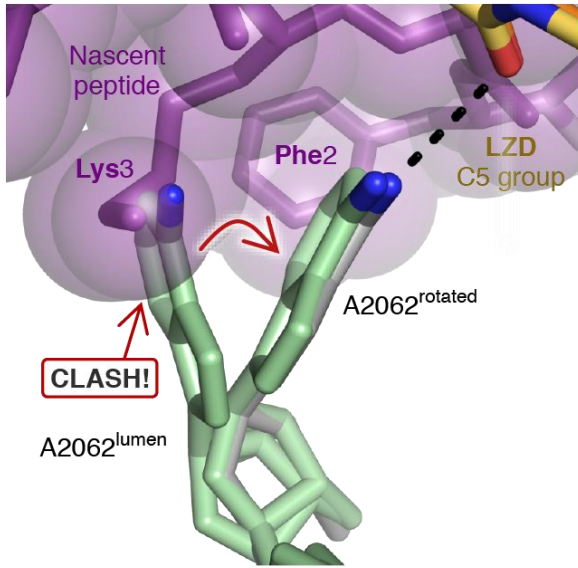


P-site
MFKAF-tRNA

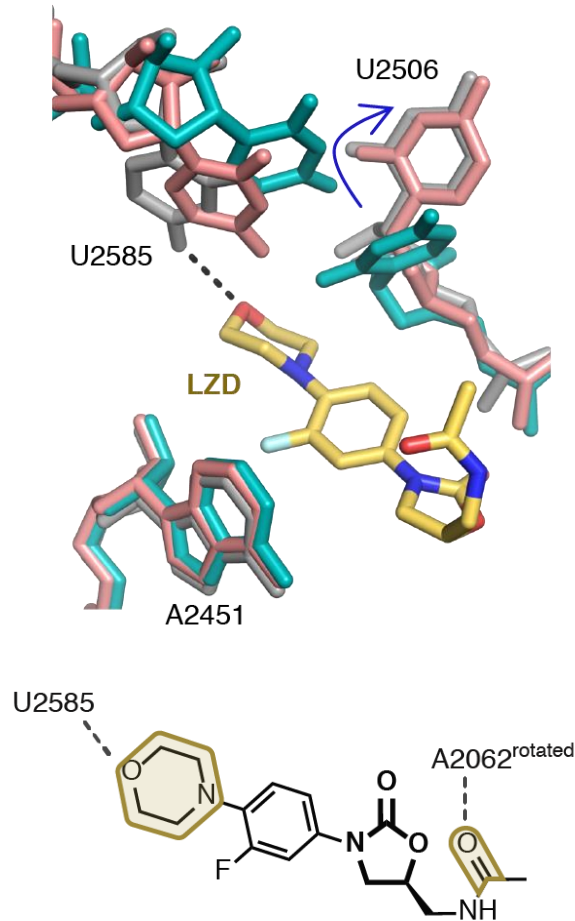
A-site



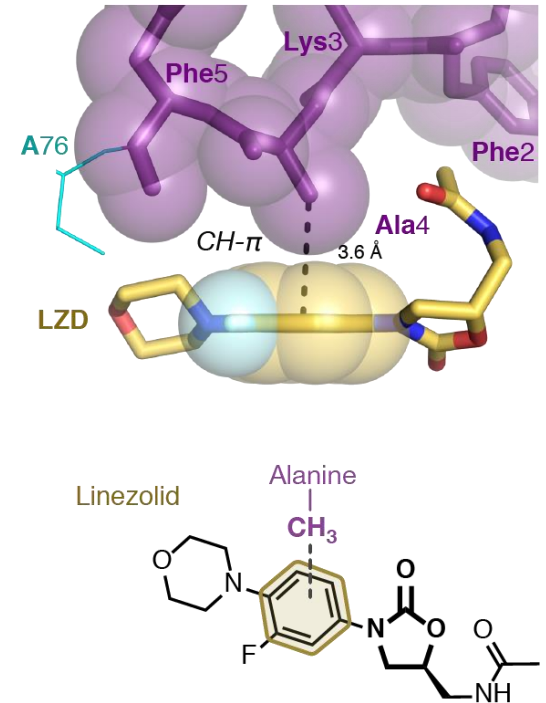
nascent protein chain
affects the rRNA structure



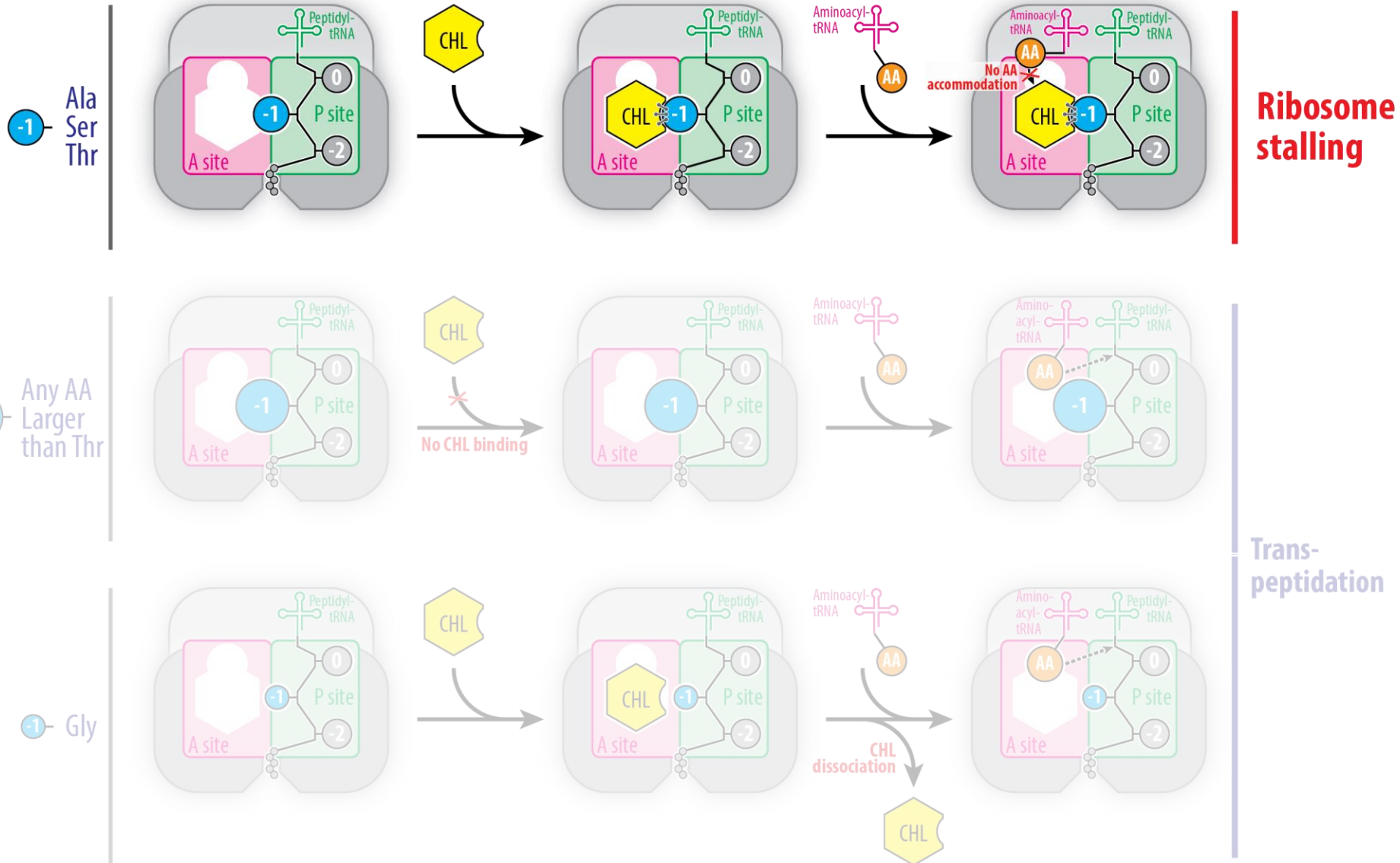
23S rRNA forms the
drug binding site



penultimate alanine
participate in creating
the high-affinity site



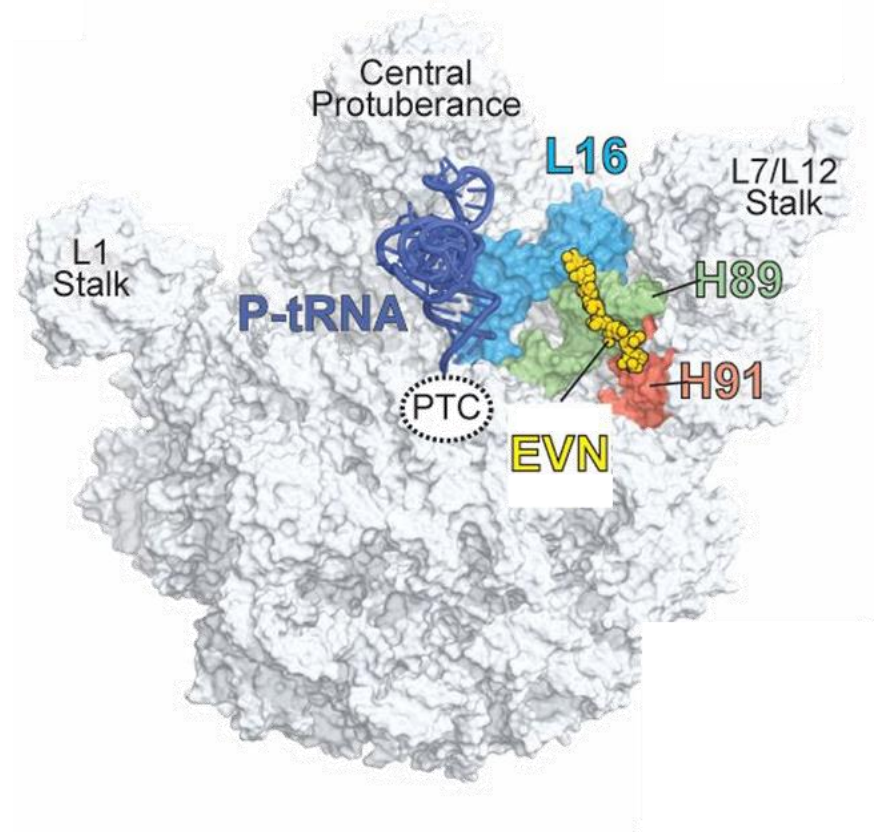
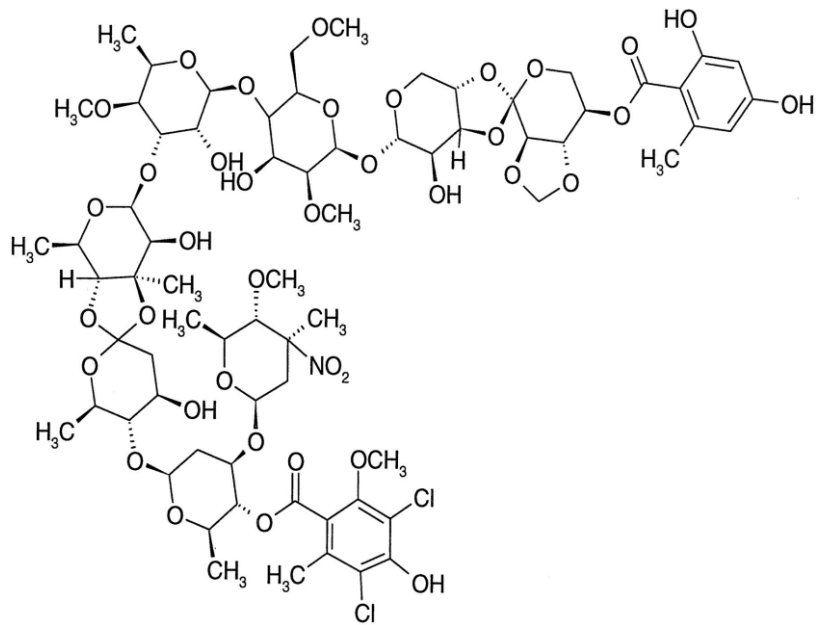
Context-specific mode of action of chloramphenicol and oxazolidinones



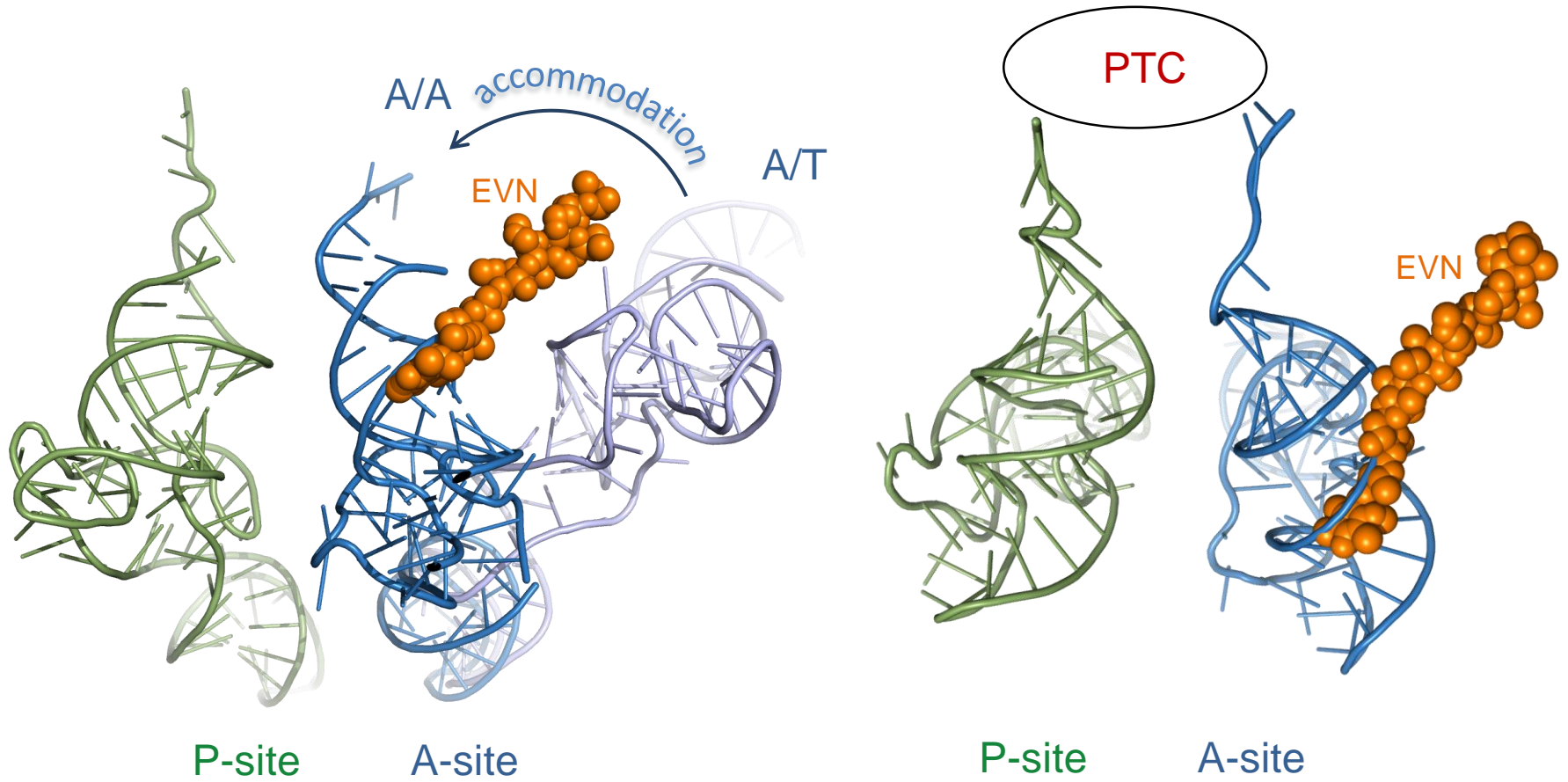
Principles of context specificity of PTC-binding phenicol and oxazolidinone antibiotics

- Nascent peptide participates in formation of the antibiotic binding site
- Context-specificity of phenicol and oxazolidinones relies on a direct interaction of the nascent peptide with the PTC-bound antibiotic

EVERNIMICIN (EVN)

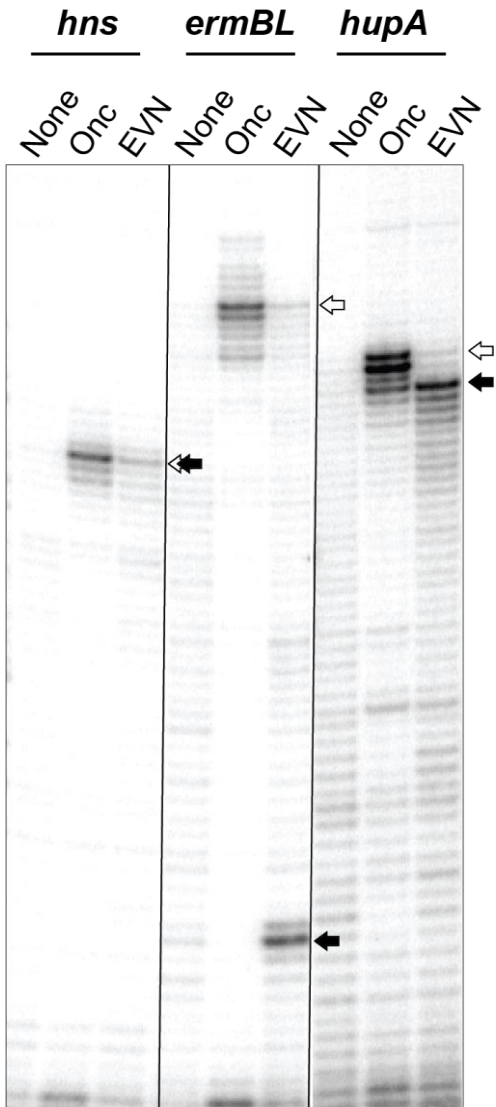


EVERNIMICIN (EVN)

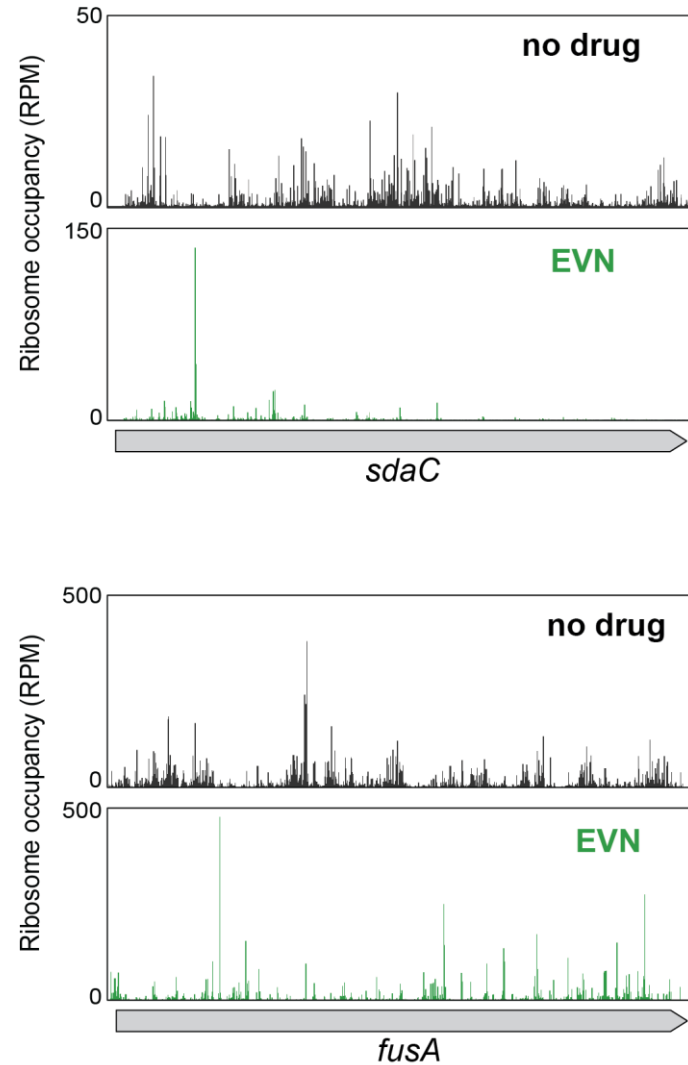


Evernimicin arrests translation at specific sites

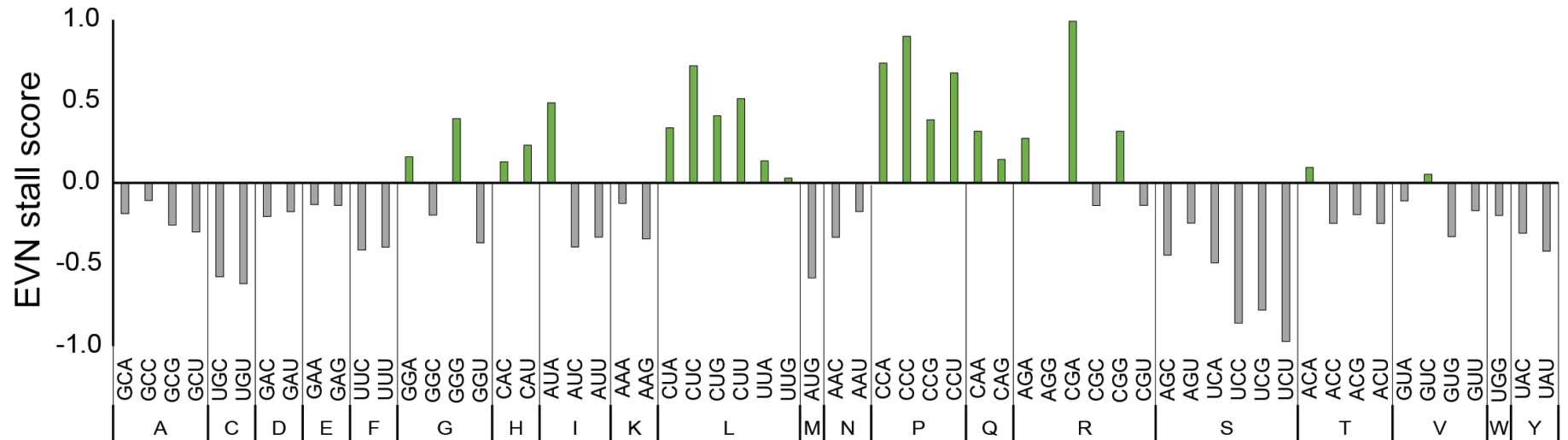
toeprinting

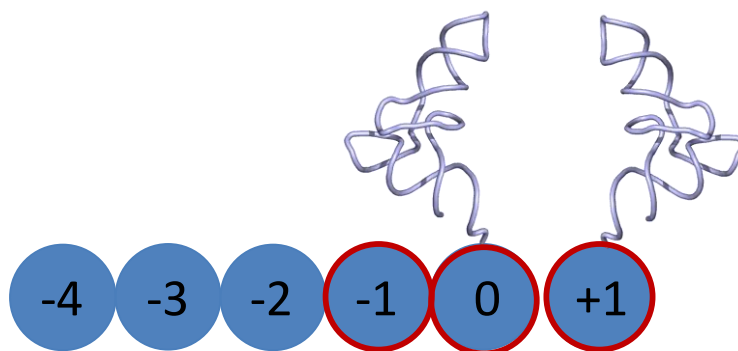
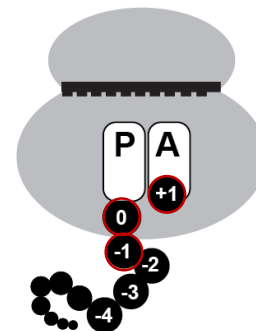
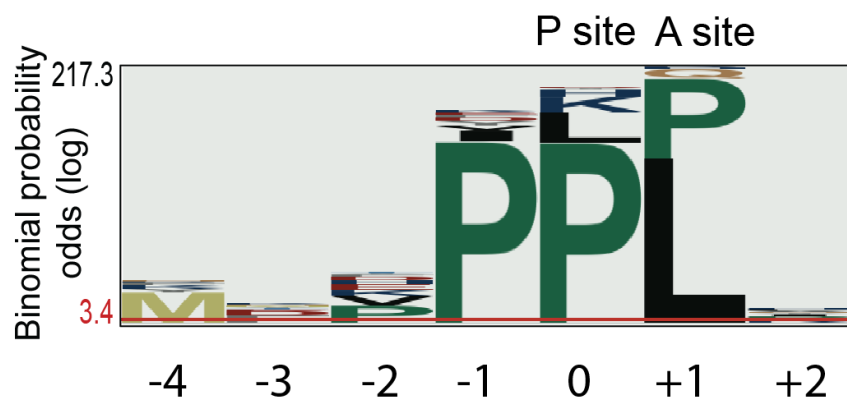
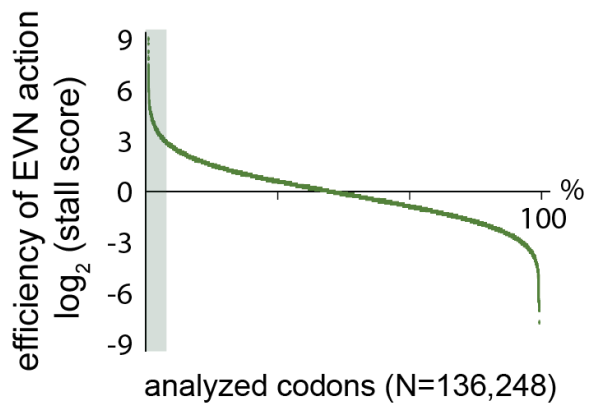


Ribo-seq

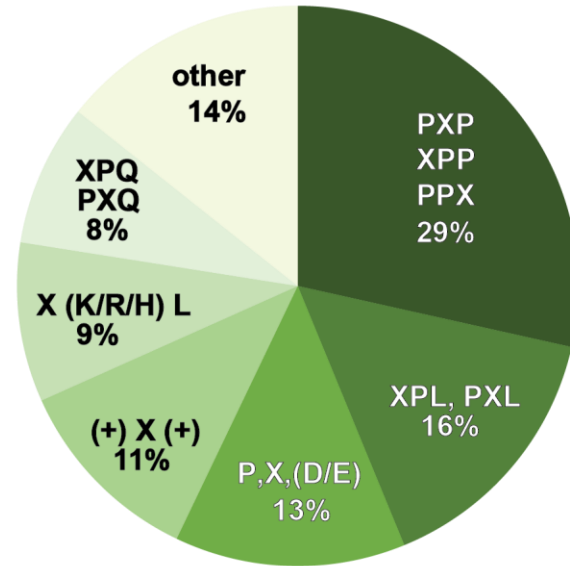
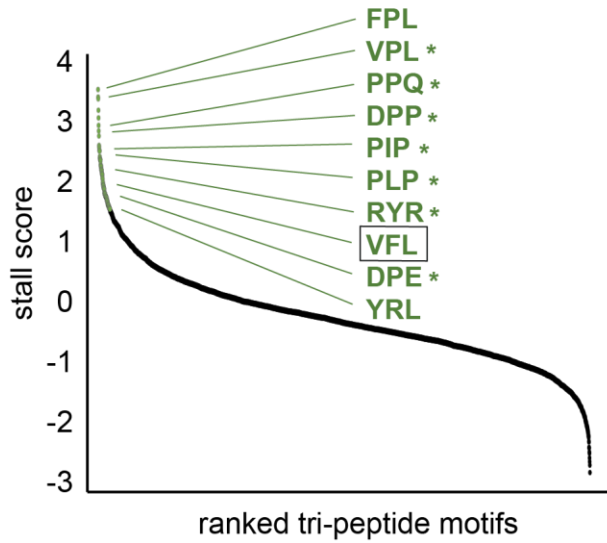


Specific A-site codons (or the incoming amino acids) are conducive to EVN action

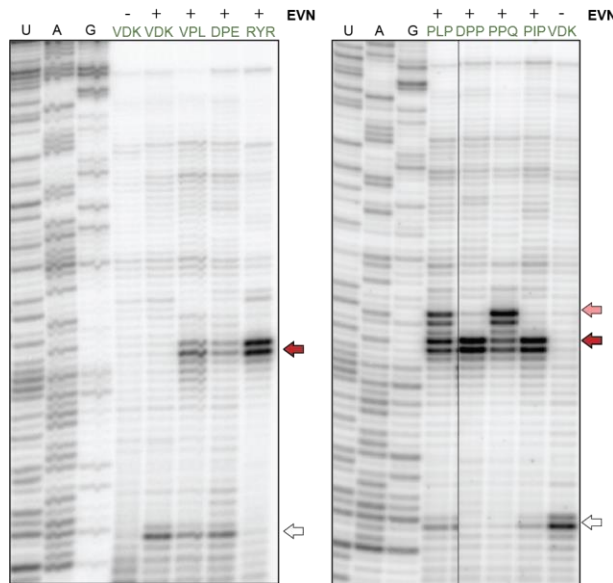




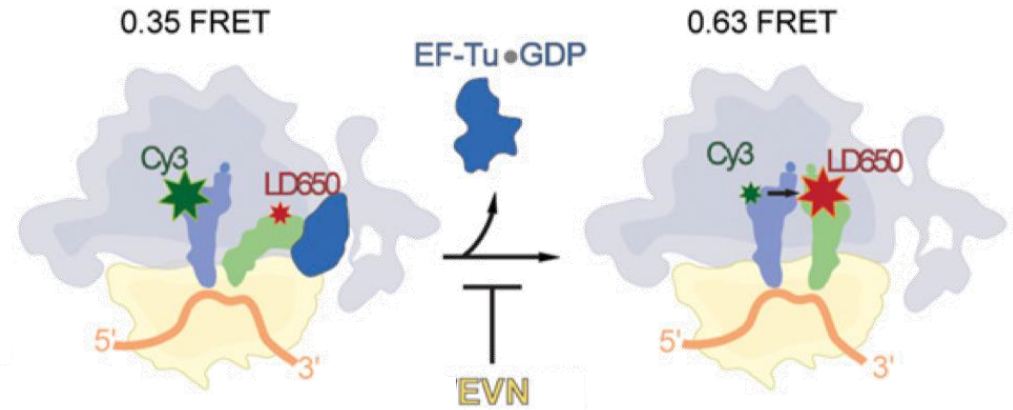
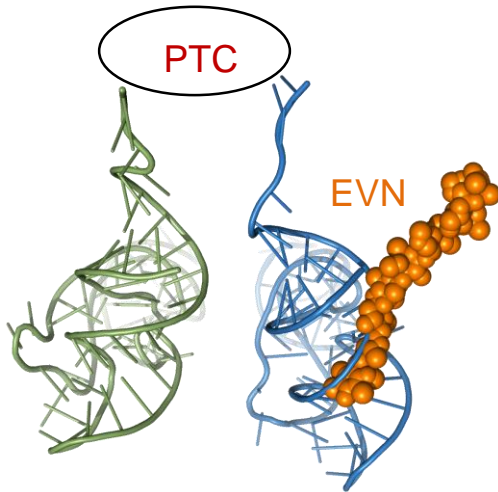
Tri-peptide motifs define the sites of evernimicin action



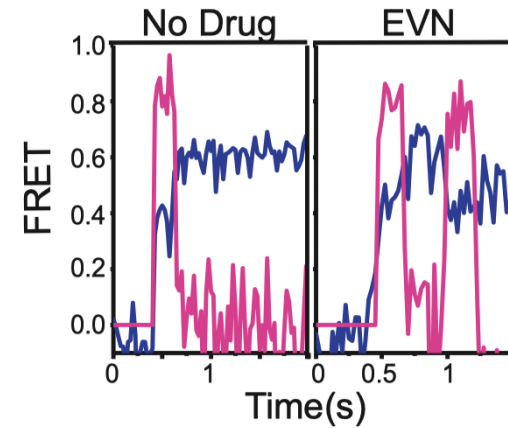
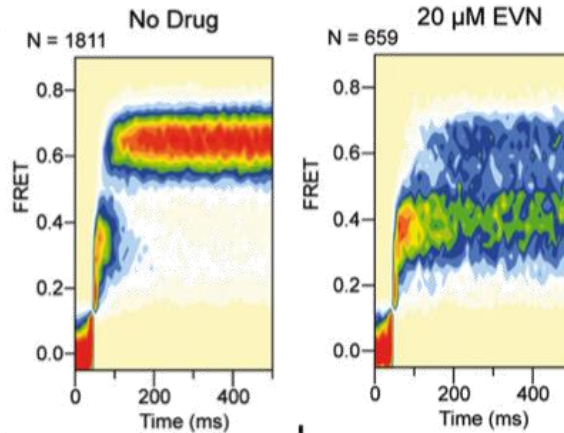
⇓ ⇓ ↓
 ErmBL: MLVFQMRN**XXX**TSTILK *



How can the nascent peptide and incoming amino acid define the site of EVN action?



P-site A-site



EVN likely allows aa-tRNA to briefly enter the PTC active site

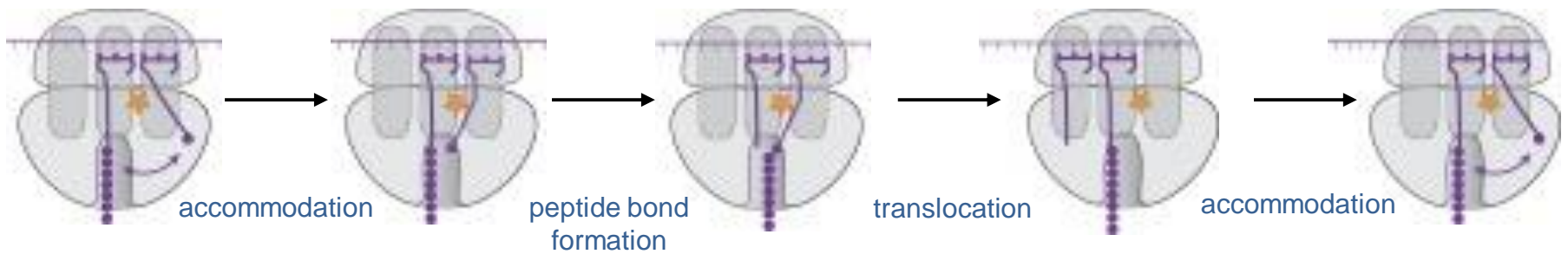
Model of site-specific action of orthosomycins

context which is conducive to the drug action

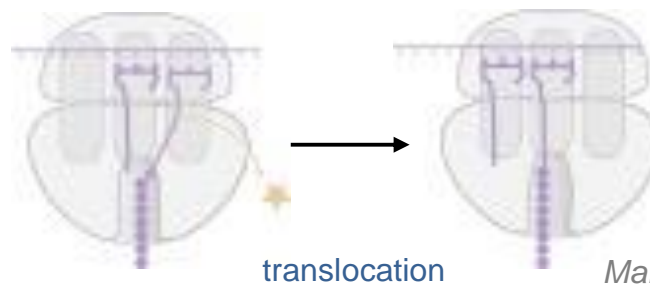
translation arrest



context which is unfavorable for the drug action



EVN ejection ↓
↑ EVN re-binding



Principle of context specificity of the accommodation corridor-binding orthosomycin antibiotics

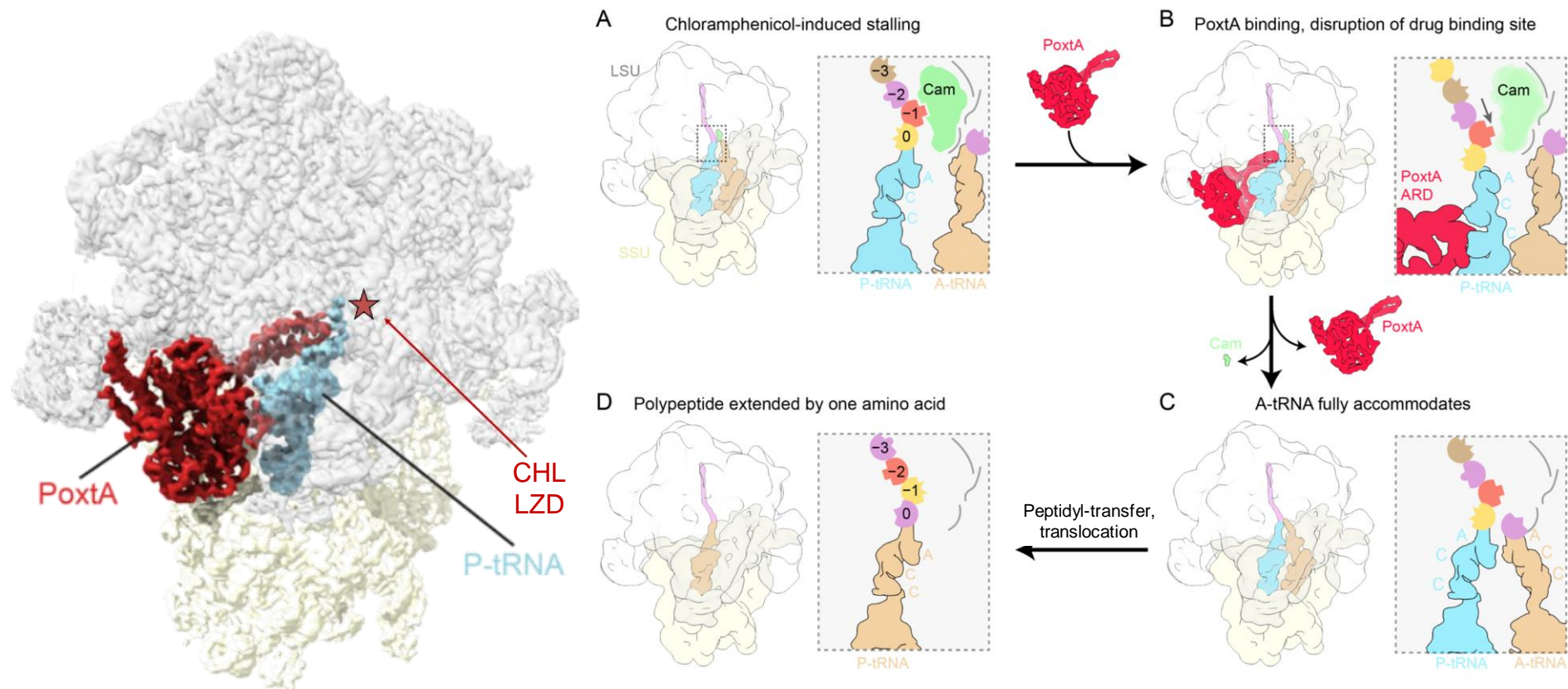
- Nascent peptide and acceptor amino acid affect the efficiency of peptide bond formation brief visit in the aa-tRNA into the PTC active site

Why should we care?

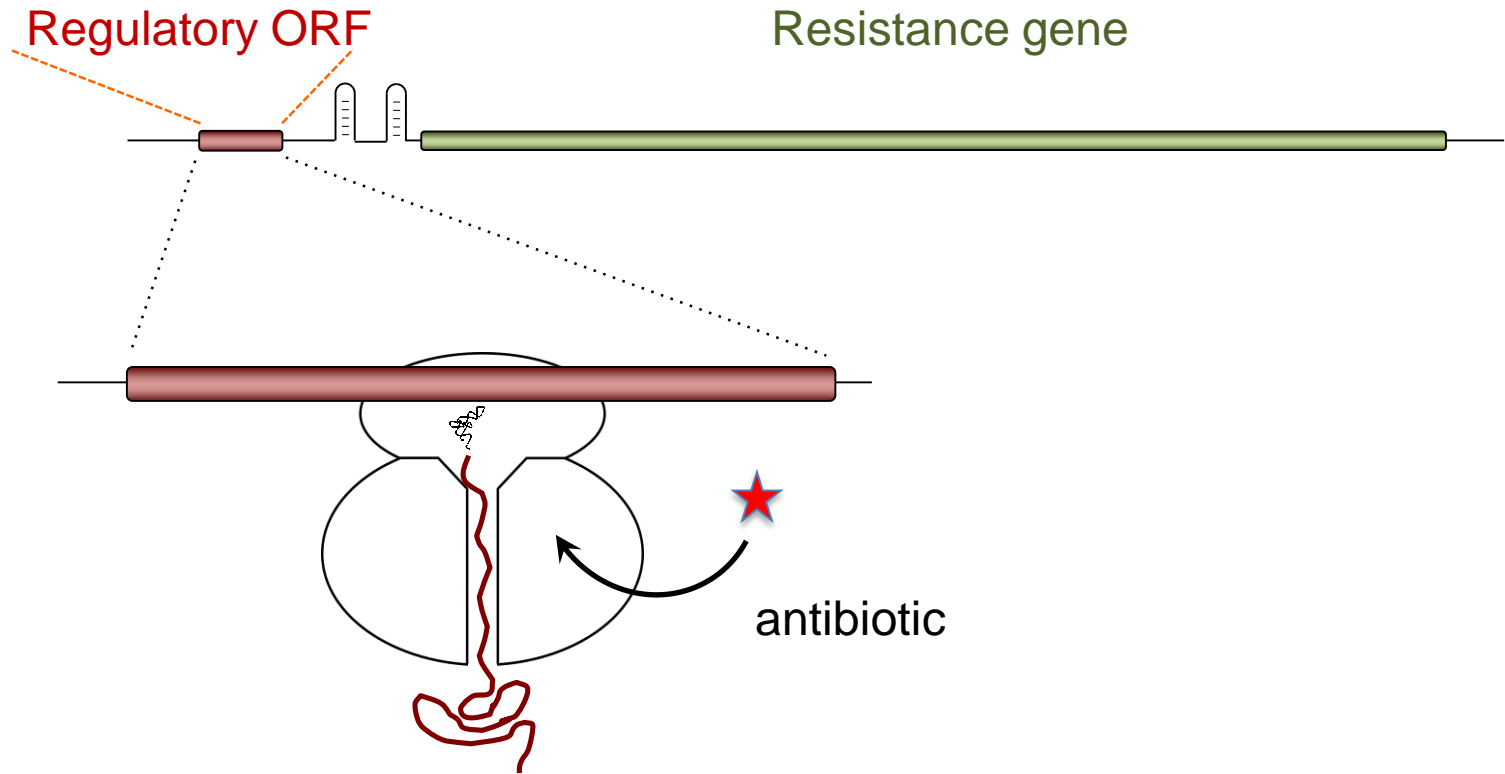
Antibiotic resistance mechanisms exploit context specificity of antibiotic action

Ribosome-protection proteins confer resistance by dislodging the antibiotic from the ribosome

PoxA renders cells resistant to CHL and LZD, but does not interact with the drug

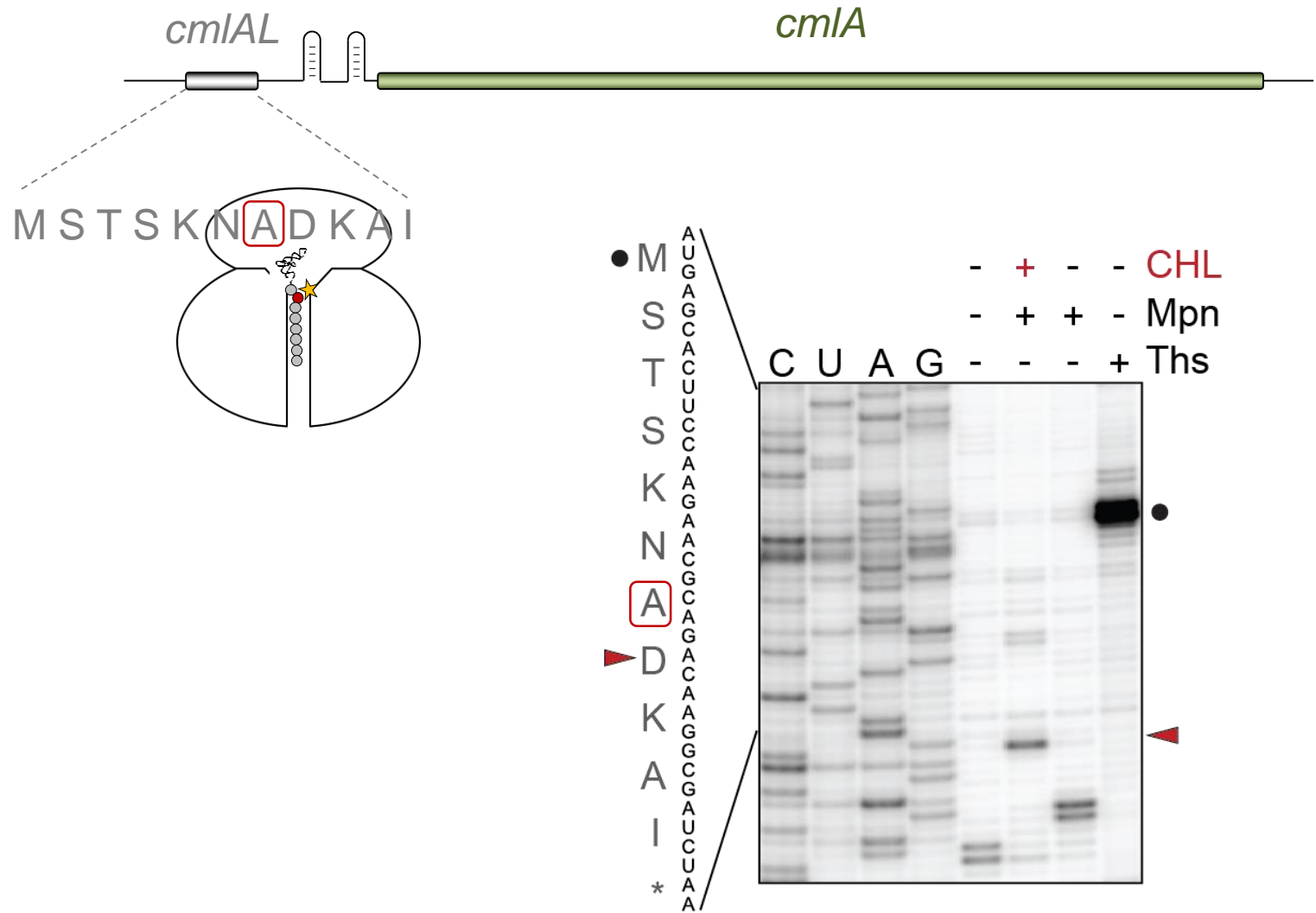


Many antibiotic resistance genes are inducible

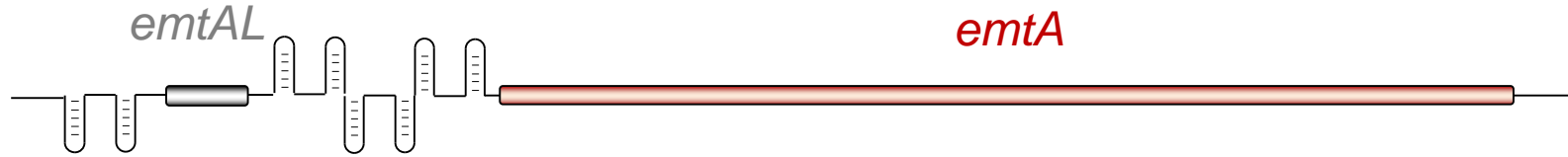


Programmed translation arrest is required for induction of resistance

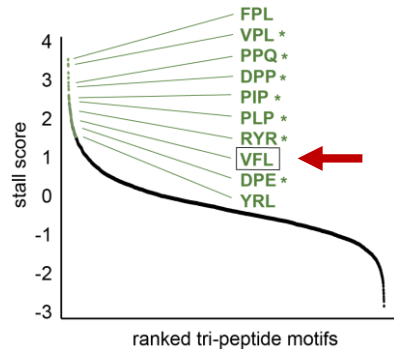
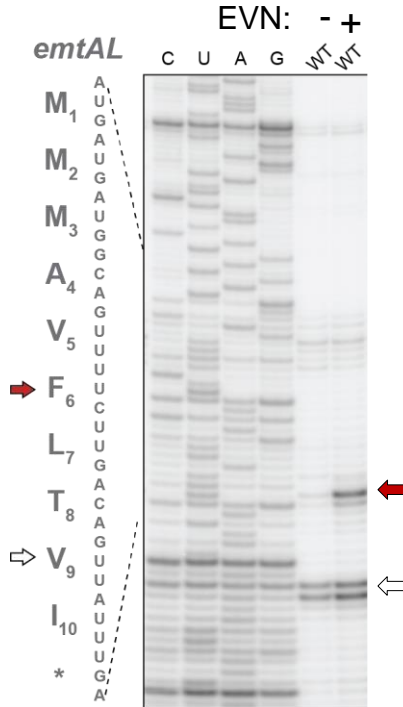
cmlA is an inducible chloramphenicol resistance gene



emtA is an rRNA methyltransferase that confers resistance to evernimicin

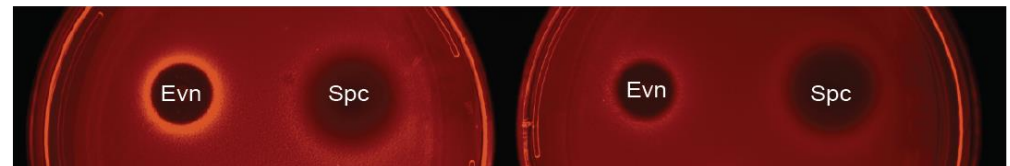


<i>Enterococcus faecium</i>	MMMAVFLT-VI
<i>Virgibacillus dokdonensis</i>	MMMAVFLT-VIR
<i>Paenactinomyces guangxiensis</i>	MMMAVFLT-VIQ
<i>Desmospora activa</i>	MKMAVFLT-VIQ
<i>Novibacillus thermophilus</i>	MMTVFLT-VIRQ
<i>Bacillus sp_SA1_12</i>	MMMMAVFLT-VIR
<i>Gracilibacillus alcaliphilus</i>	MKMAVFLA-VIR
<i>Paenactinomyces guangxiensis</i>	MMMAVFLT-VIQ
<i>Seionella peptonophila</i>	MKMAVFLI-VIQ
<i>Paenibacillus sp_320_W</i>	MMAVFLTVCVHSN
<i>Paenibacillus hemerocallicola</i>	MMMAVFLT VVVIS
<i>Paenibacillus mesophilus</i>	MMMAVFLT VVVIS
<i>Paenibacillus forsythiae</i>	MMMAVFLT VVIQ
<i>Paenibacillus sp_VKM_B_2647</i>	MMMTVFLTVAIG
<i>Paenibacillus favisporus</i>	MMMMTVFLT IHSVN
<i>Paenibacillus flagellatus</i>	MMMMTVFLTAVIR
<i>Paenibacillus nasutitermitis</i>	MMMMAVFLT VVQFS
<i>Cohnella nanjingensis</i>	MMMTVFLAVVIG
<i>Melghirimyces algeriensis</i>	MKMAVFLTDDSVILN
<i>Paludifilum halophilum</i>	MKMAVFLADGSVI



MMMAVFL

MMMAVFA



Conclusions

- Many (possibly most and maybe all) ribosomal antibiotics act in a context-specific manner.
- Determinants of specificity often reside in the sequence of the nascent protein chain
- The incoming acceptor amino acid may critically affect the extent of translation arrest imposed by antibiotic
- Unraveling context specificity of ribosome-targeting antibiotics is critical for understanding their mode of action and operation of the resistance mechanisms

ACKNOWLEDGEMENTS



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Kyle Mangano



James Marks



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Teresa Szal
Sezen Meydan
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Lund University

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