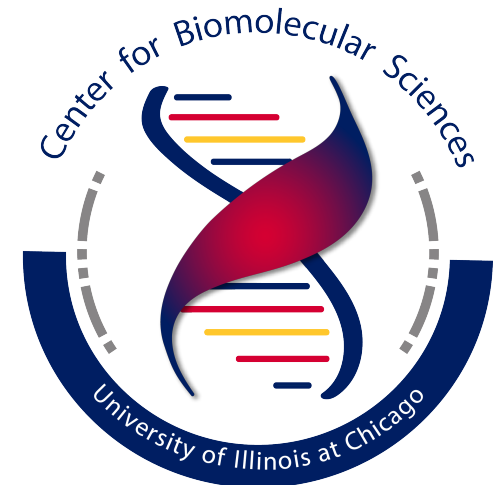
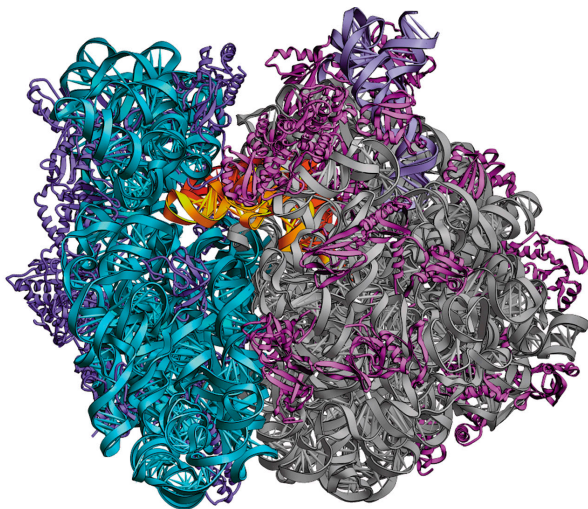
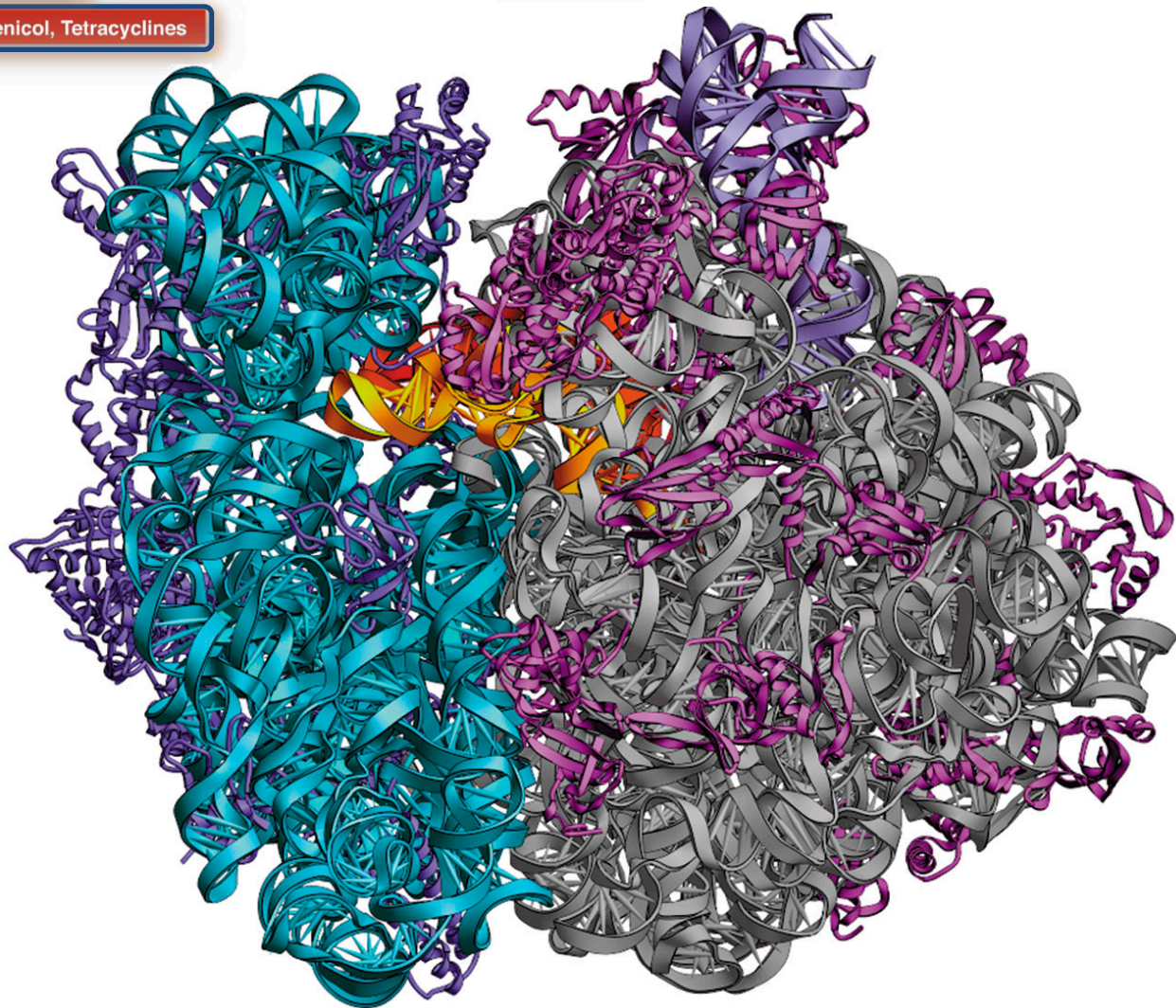
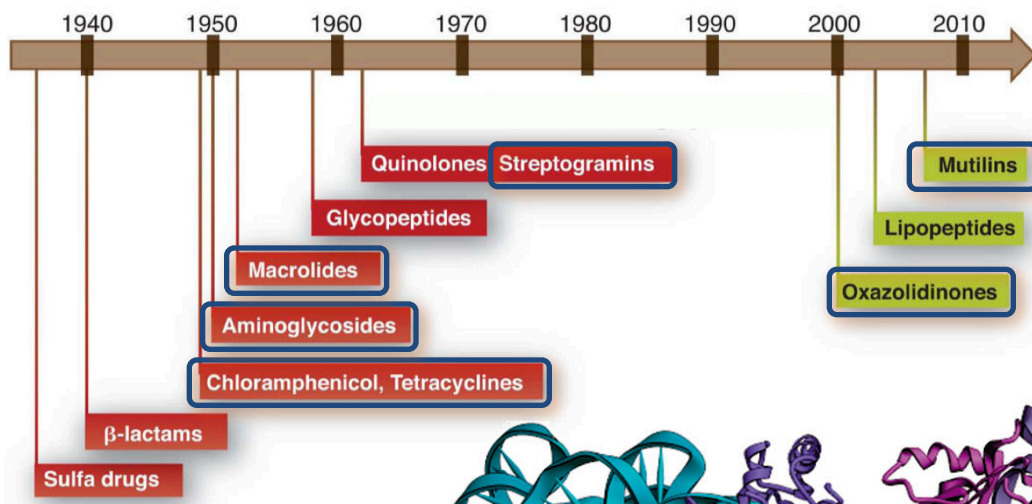


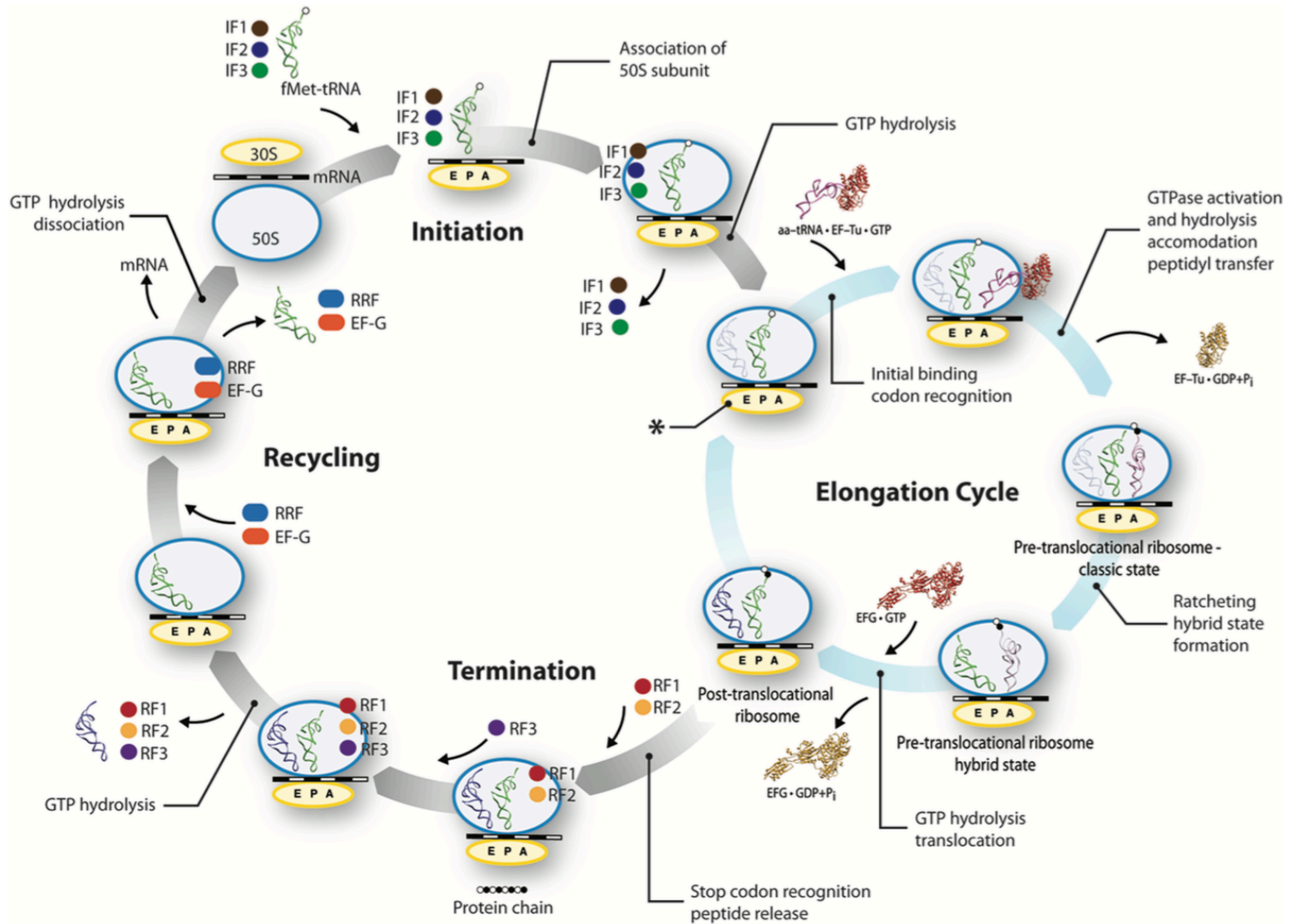
Context-specific modulation of translation by ribosome-targeting antibiotics

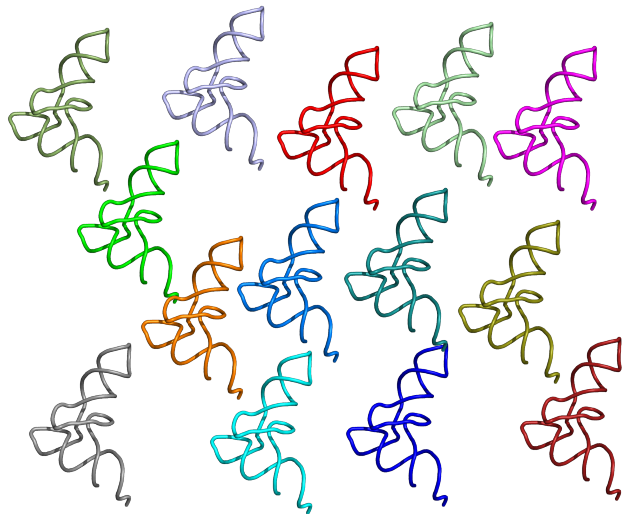
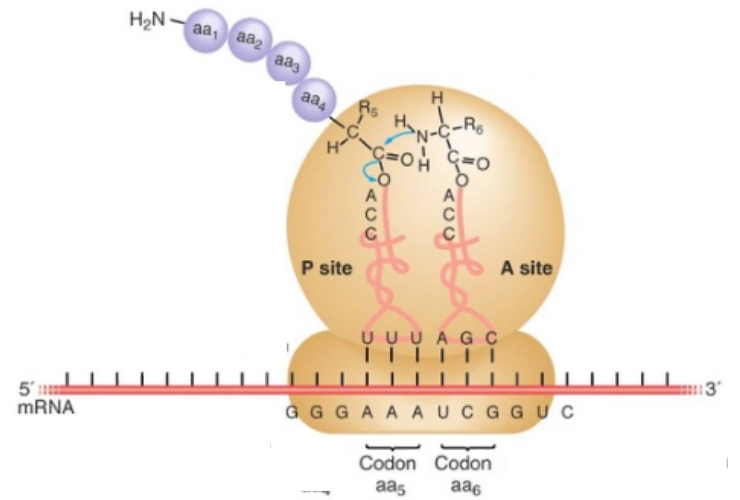
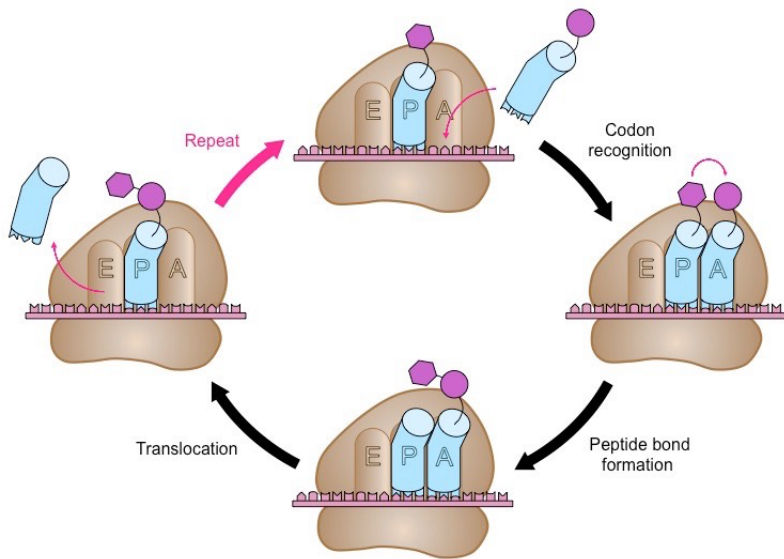
Alexander Mankin



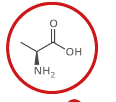
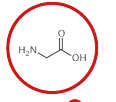
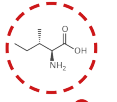
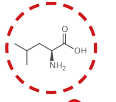
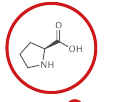
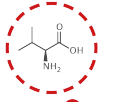
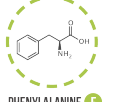
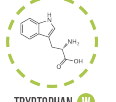
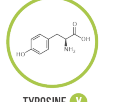
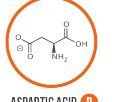
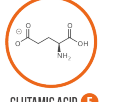
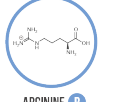
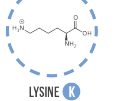
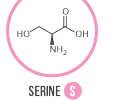
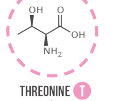
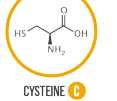
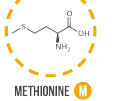
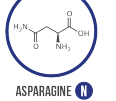
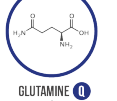


Ribosome interacts with many different ligands during protein synthesis





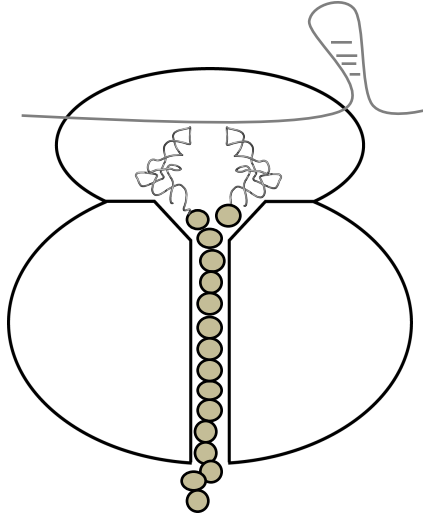
● ALIPHATIC
 ● AROMATIC
 ● ACIDIC
 ● BASIC
 ● HYDROXYLIC
 ● SULFUR-CONTAINING
 ● AMIDIC
 ○ NON-ESSENTIAL
 ⊕ ESSENTIAL

 ALANINE (A) <i>ala</i>	 GLYCINE (G) <i>gly</i>	 ISOLEUCINE (I) <i>ile</i>	 LEUCINE (L) <i>leu</i>	 PROLINE (P) <i>pro</i>	 VALINE (V) <i>val</i>
 PHENYLALANINE (F) <i>phe</i>	 TRYPTOPHAN (W) <i>trp</i>	 TYROSINE (Y) <i>tyr</i>	 ASPARTIC ACID (D) <i>asp</i>	 GLUTAMIC ACID (E) <i>glu</i>	 ARGININE (R) <i>arg</i>
 LYSINE (K) <i>lys</i>	 SERINE (S) <i>ser</i>	 THREONINE (T) <i>thr</i>	 CYSTEINE (C) <i>cys</i>	 METHIONINE (M) <i>met</i>	 ASPARAGINE (N) <i>asn</i>
					 GLUTAMINE (Q) <i>gln</i>

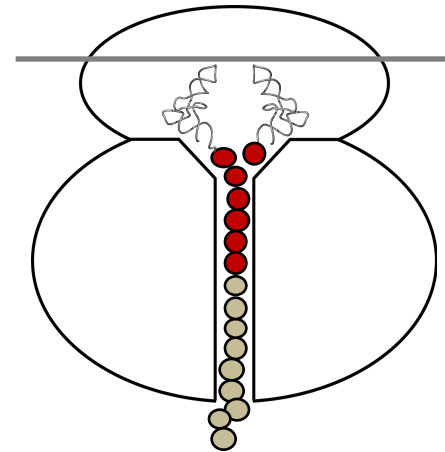
1 initiator and 48 elongator tRNAs in *E. coli*:
2352 combinations of the P and A site tRNAs

400 combinations
of donor and acceptor amino acids

mRNA structure affects the ribosome progression



Nascent protein chain can slow elongation



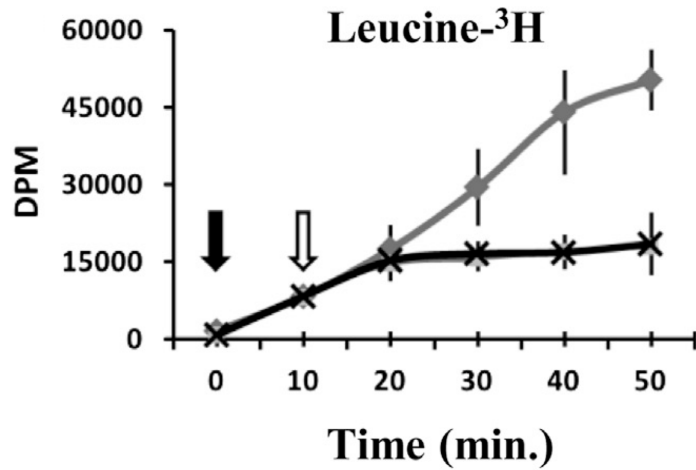
The ribosome can be paused in specific conformations either more prone or more refractory to antibiotic binding

Different states of the ribosome must be differentially affected by
antibiotics

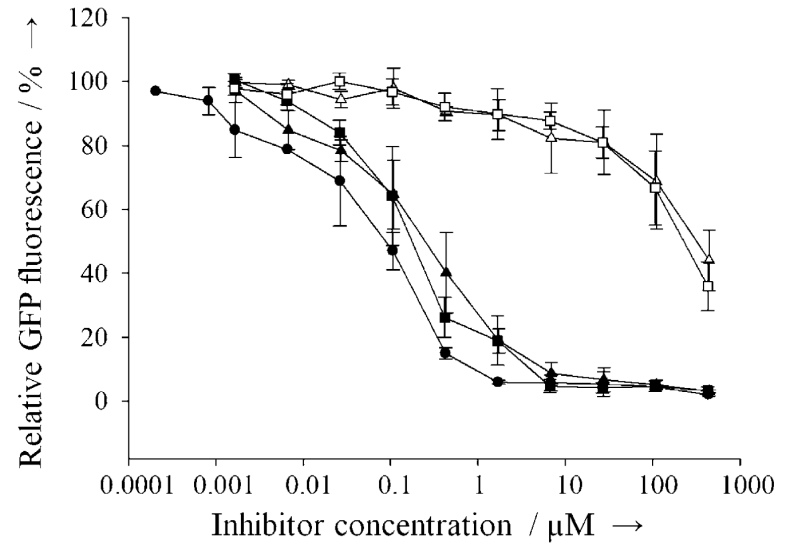
Why context specificity of ribosomal antibiotics was not discovered earlier?

Traditional techniques

In vivo

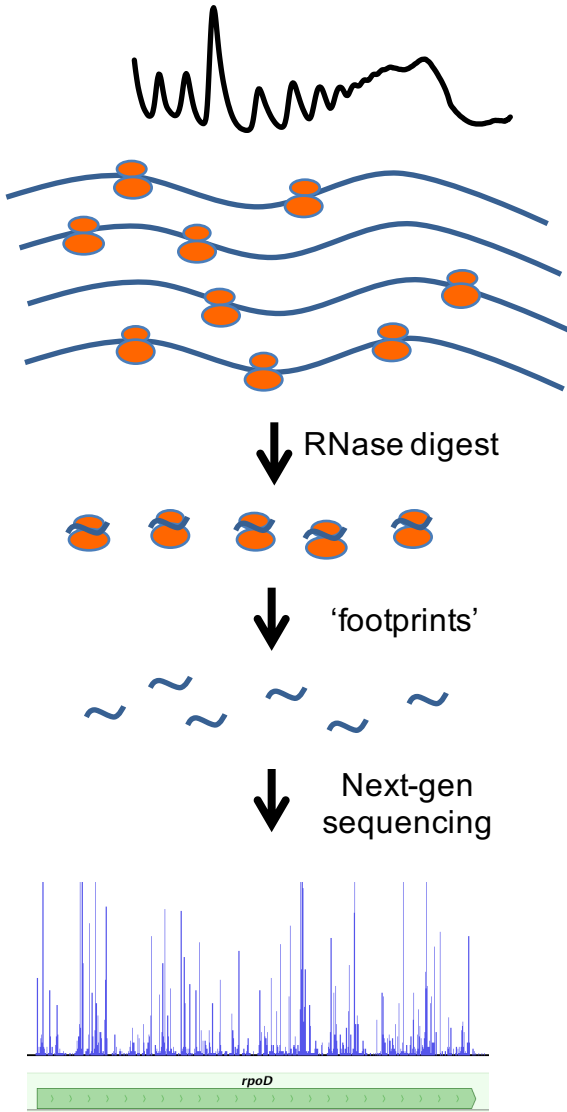


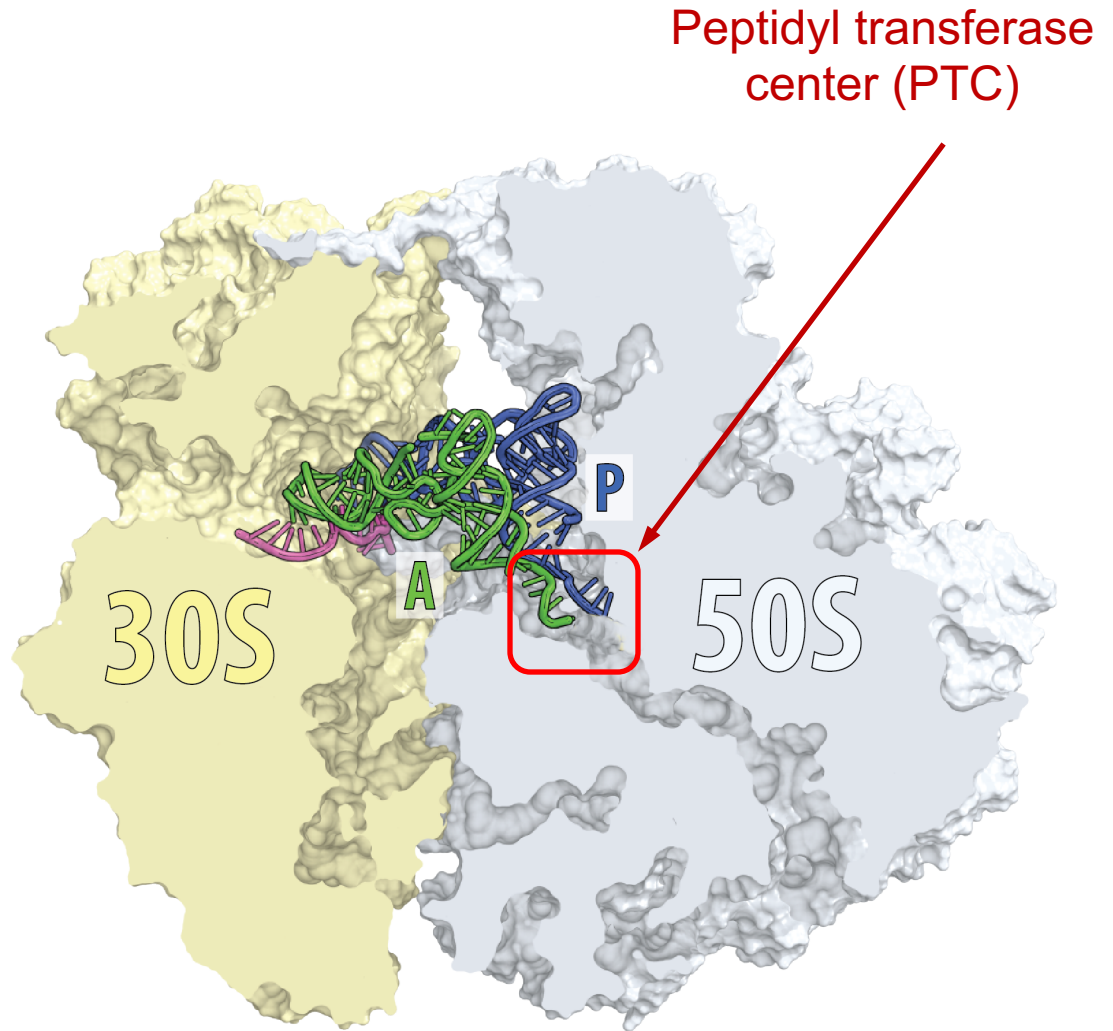
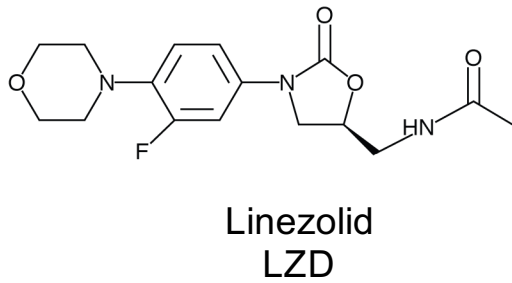
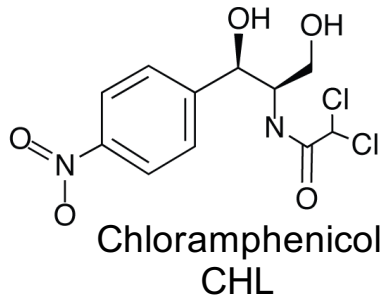
In vitro



poly(U), poly(A), lacZ, gfp, luc

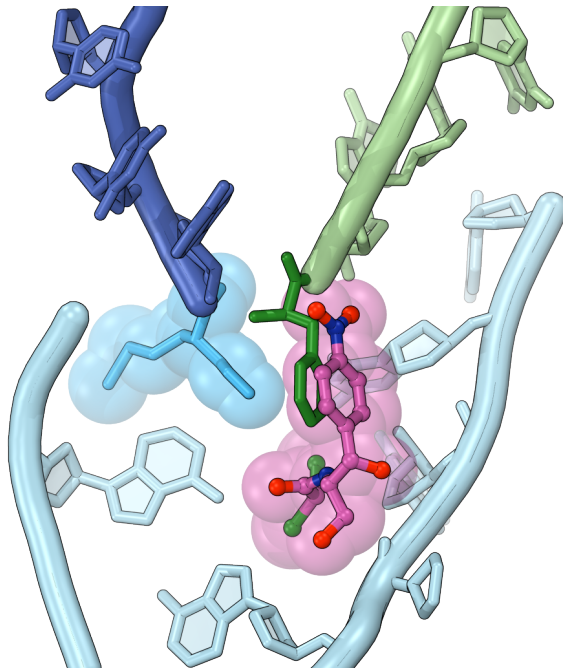
Genome-wide analysis of antibiotic action can reveal gene- and codon-specificity





P-tRNA

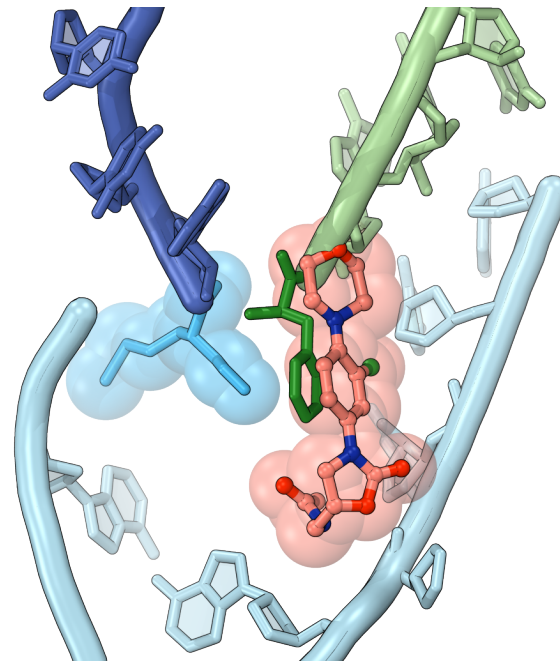
A-tRNA



Chloramphenicol

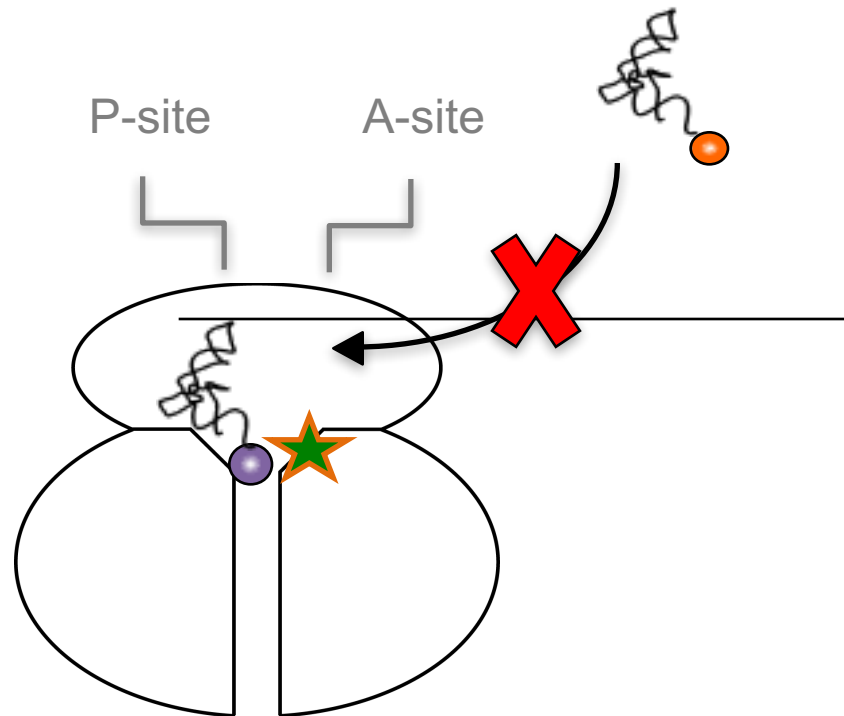
P-tRNA

A-tRNA

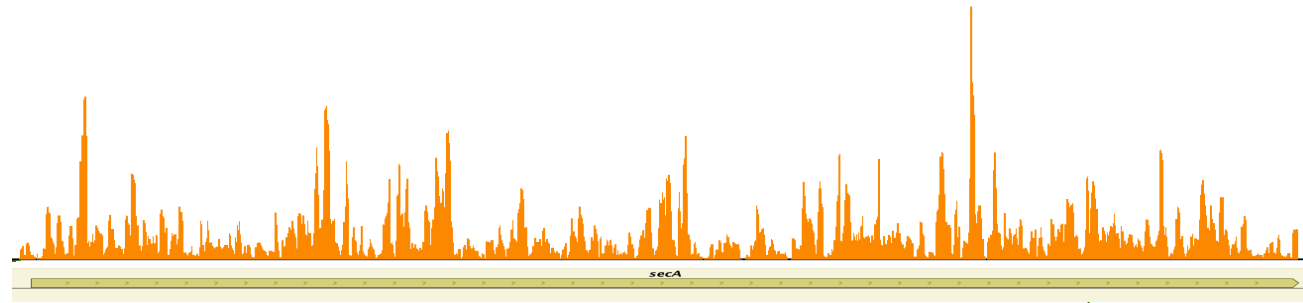


Linezolid

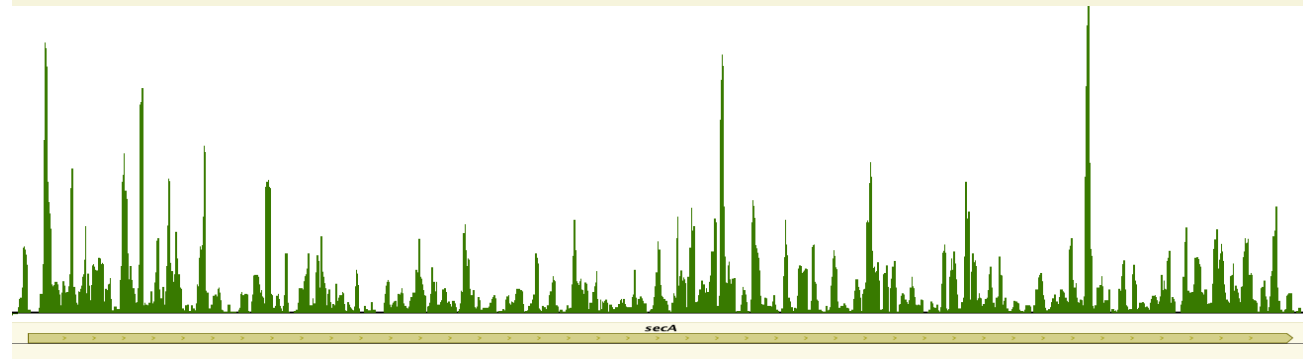
CHL and LZD should inhibit formation of **every** peptide bond because they should compete with **any** aminoacyl-tRNA



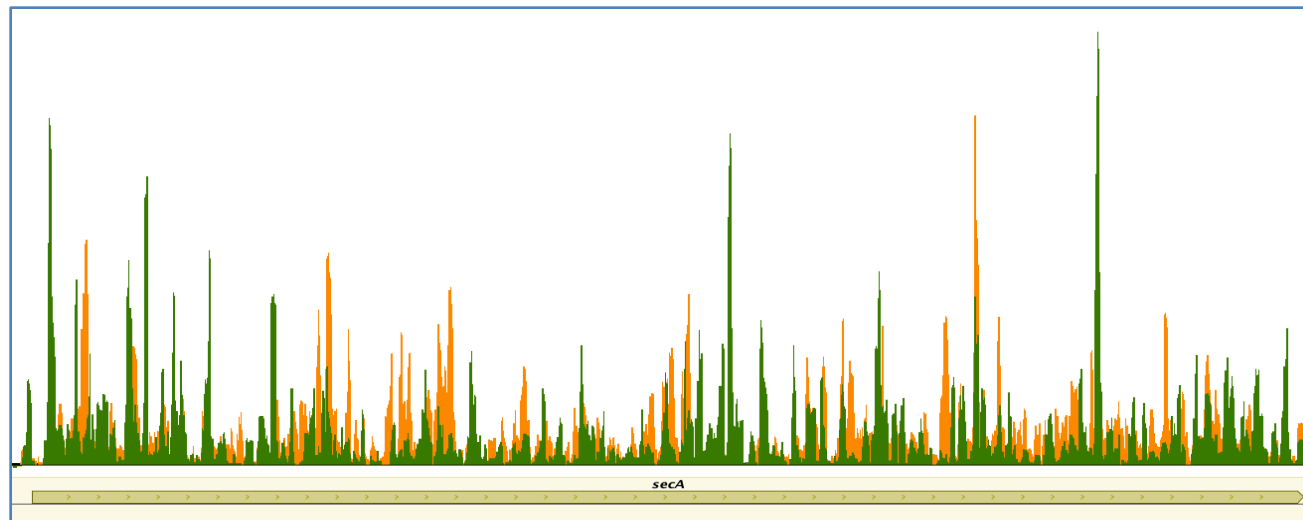
CHL and LZD cause redistribution of ribosomes on mRNAs



no drug



chloramphenicol

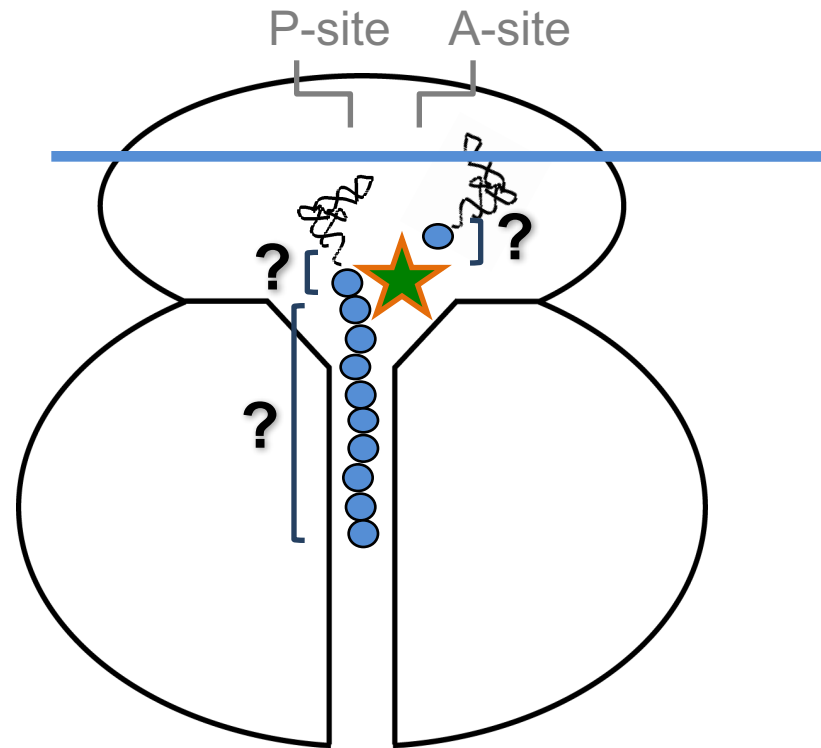
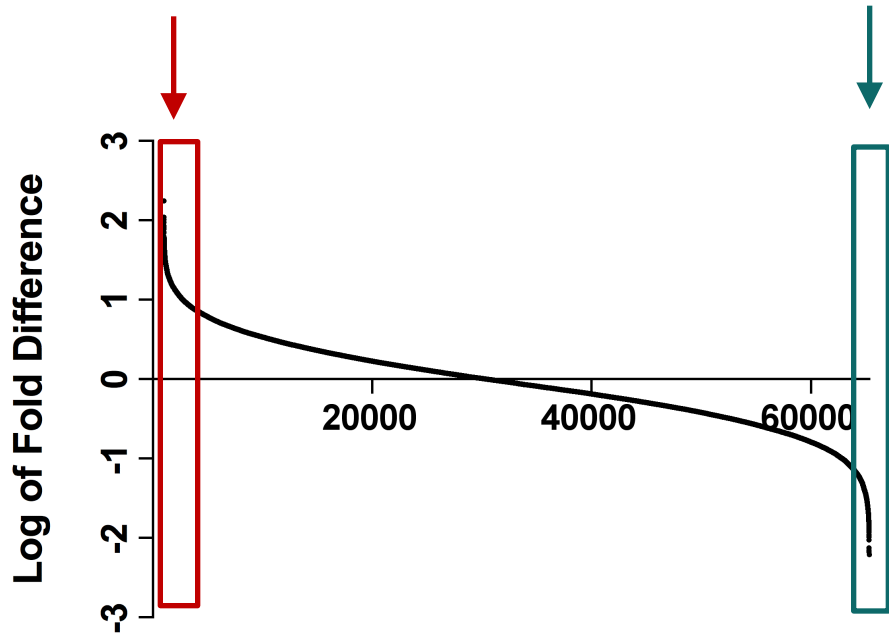


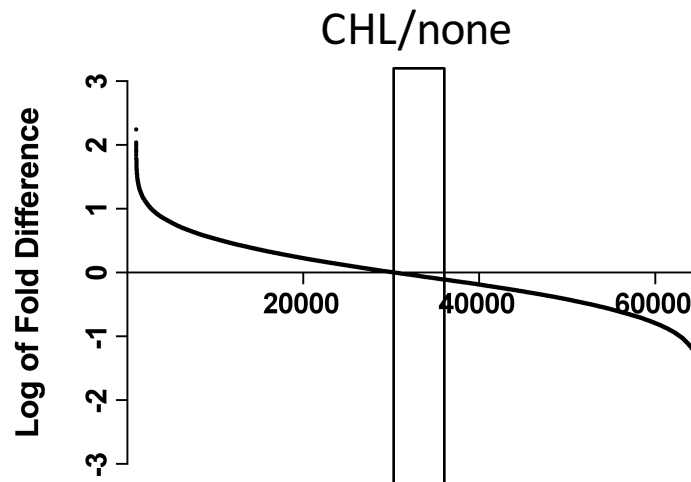
What are the sites where CHL and LZD arrest translation
in the cell?

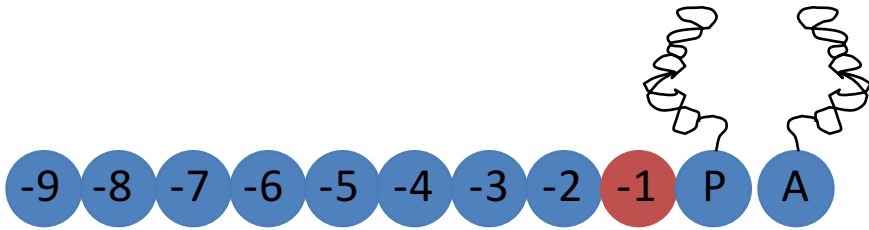
What distinguishes the sites of the preferential antibiotic action?

Strong antibiotic action

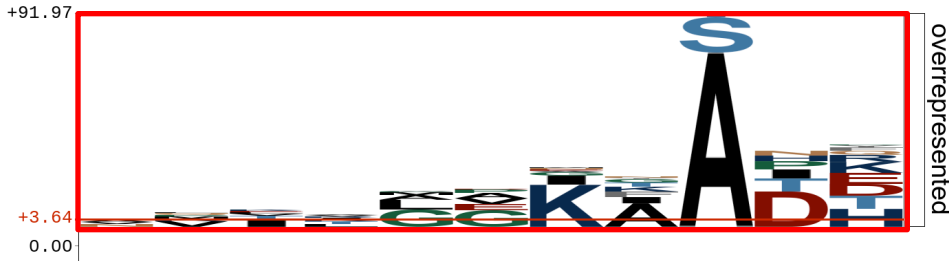
Weak antibiotic action



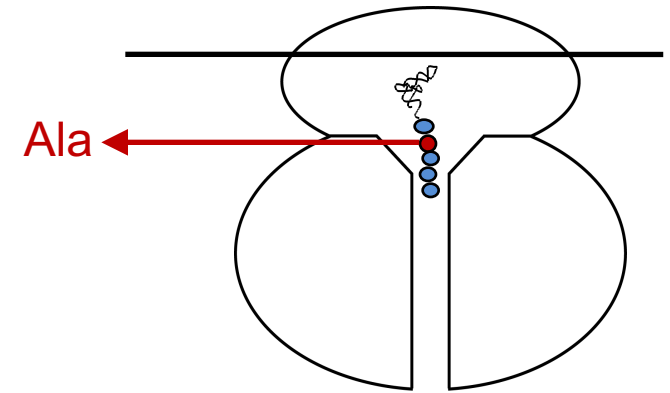
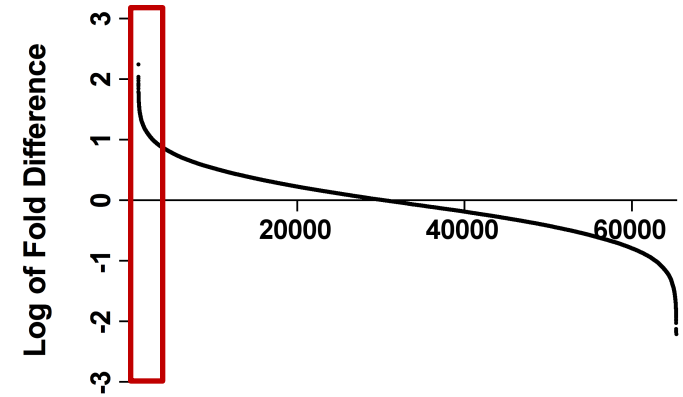




CHL

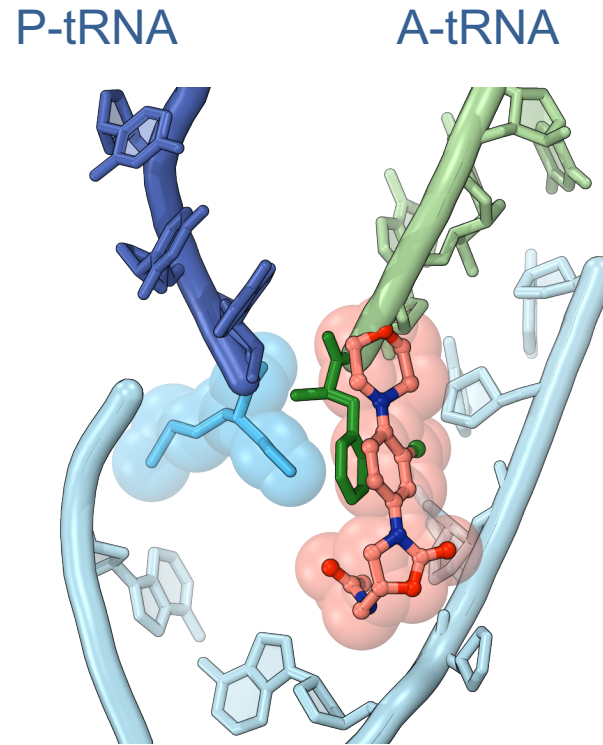


LZD



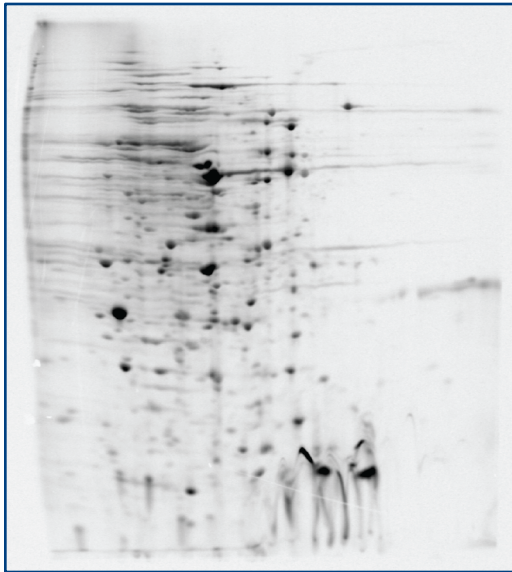
Linezolid and chloramphenicol preferentially inhibit translation when Ala is in the penultimate position of the nascent protein

Penultimate amino acid of the nascent chain can directly interact with the antibiotic



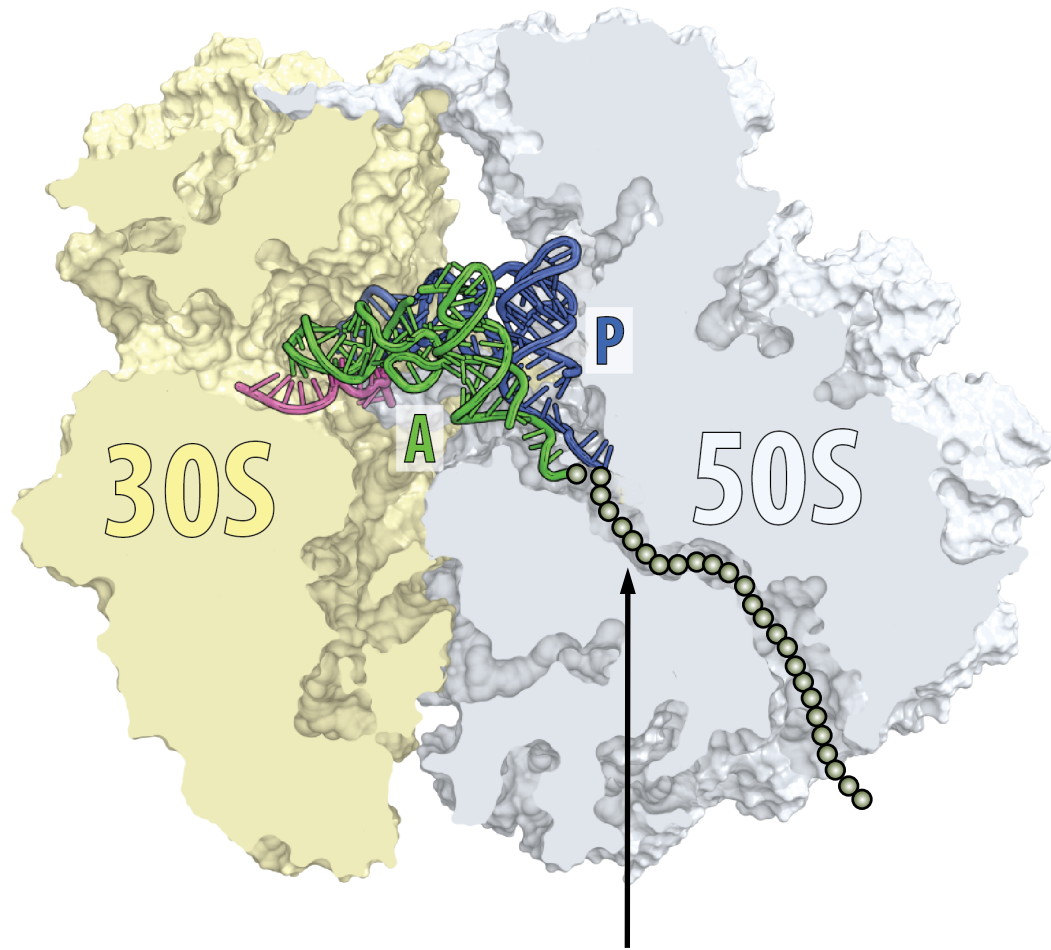
Most of the proteins contain alanine, serine or threonine.
At a high concentration of CHL or LZD, synthesis of all proteins is inhibited

no drug



linezolid

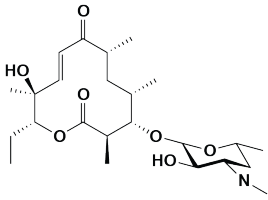




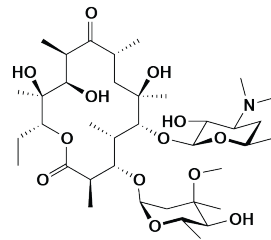
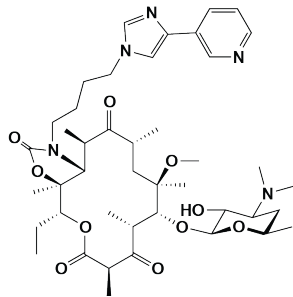
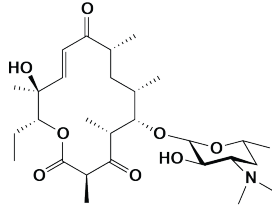
Nascent peptide
exit tunnel

Macrolides bind at the nascent peptide exit tunnel

Methymycin
(MTM)

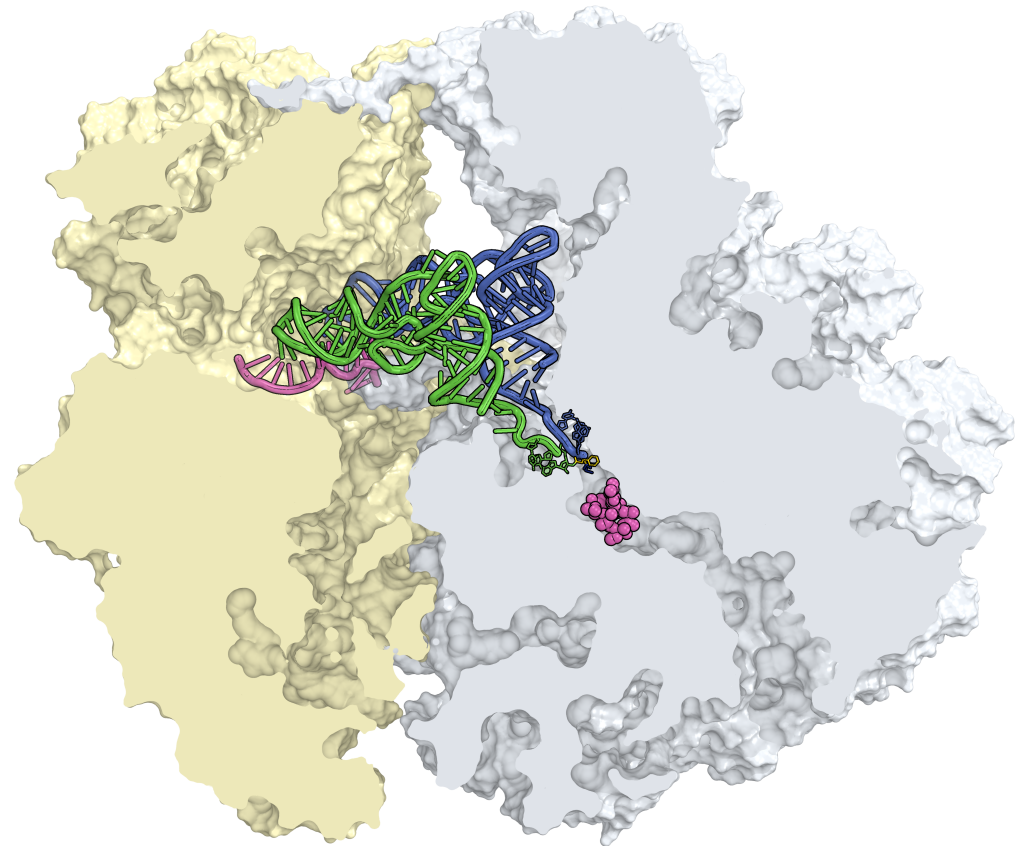


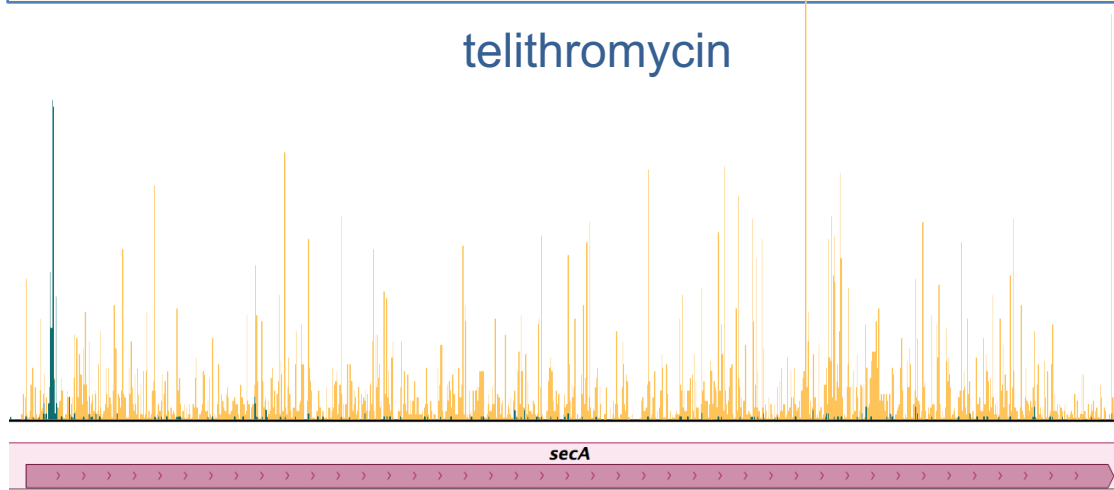
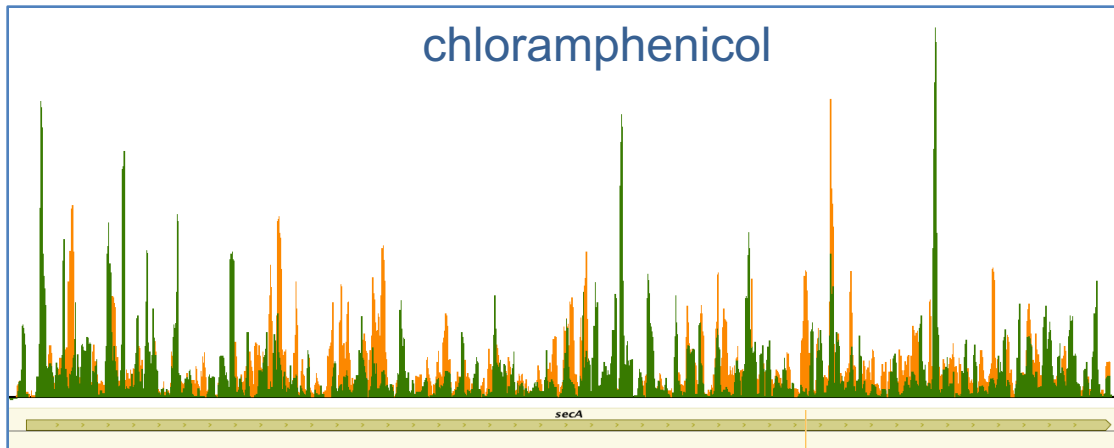
Pikromycin
(PKM)

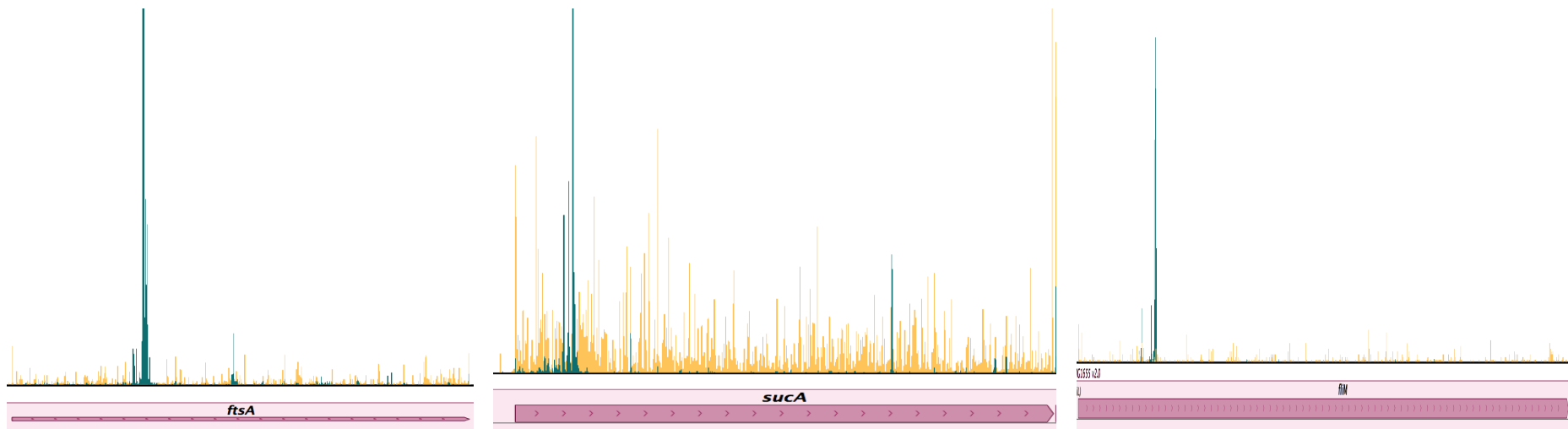


Telithromycin
(TEL)

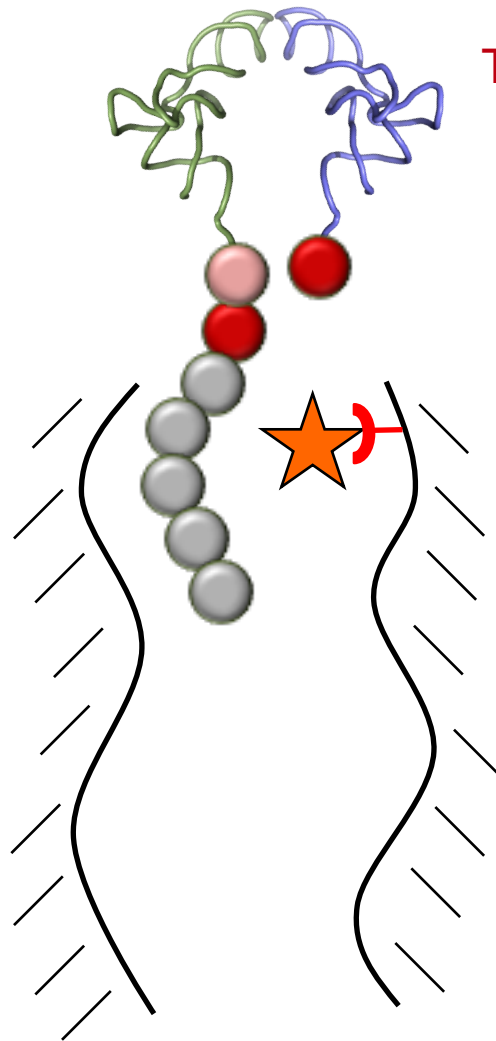
Erythromycin
(ERY)







Macrolides arrest translation at a limited number of sites within the gene.
 Specific sequence motifs define the sites of the macrolide action.

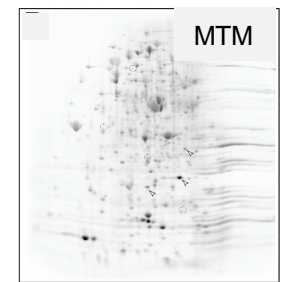
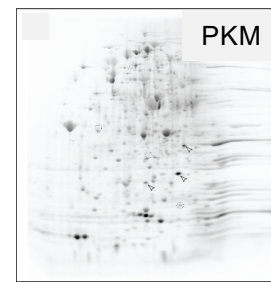
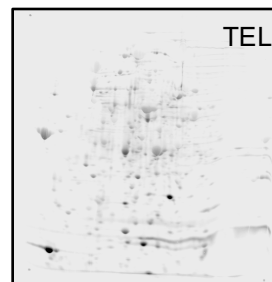
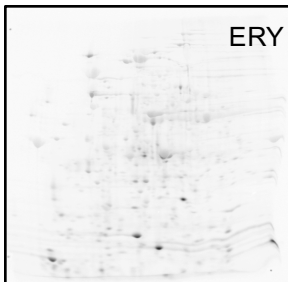
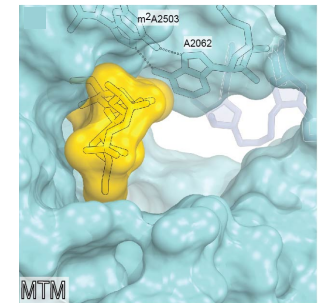
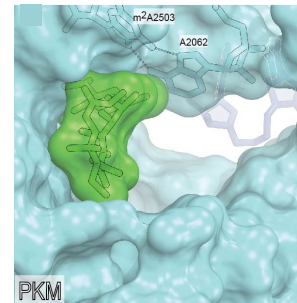
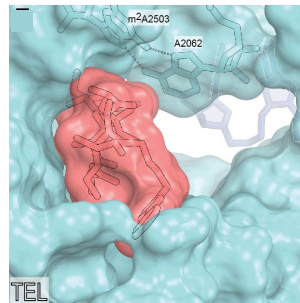
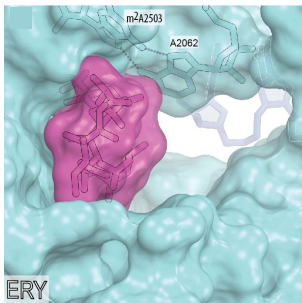
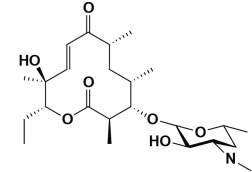
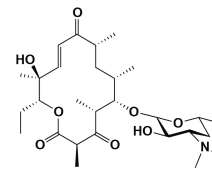
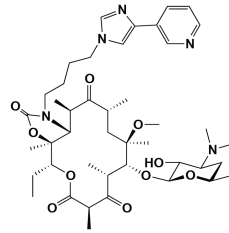
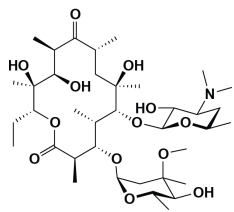
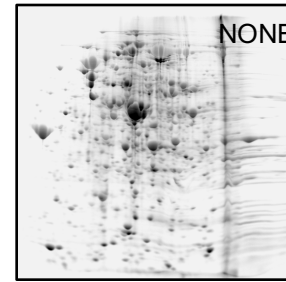
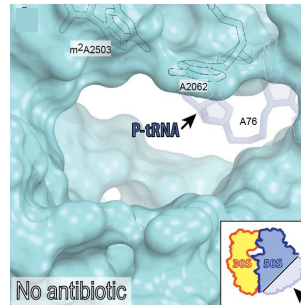


The predominant motif for the
macrolide action:

$[R/K] \times [R/K]$

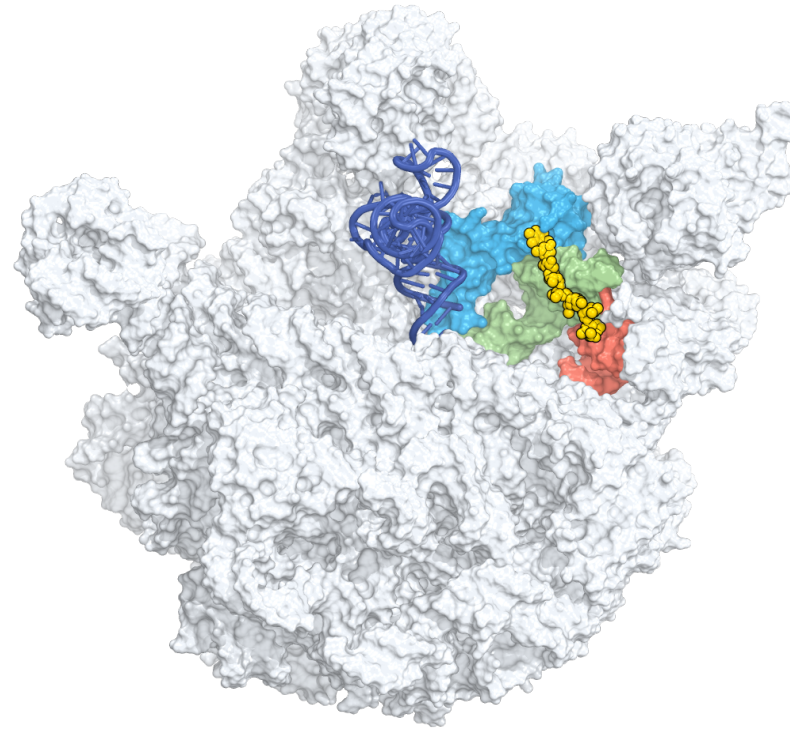
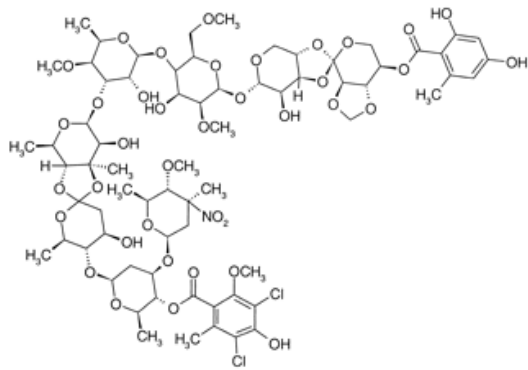
C-terminus of the nascent chain and the incoming amino acid
are critical for the main 'problematic' motif.

The structure of the antibiotic determines the protein selectivity of the inhibitor

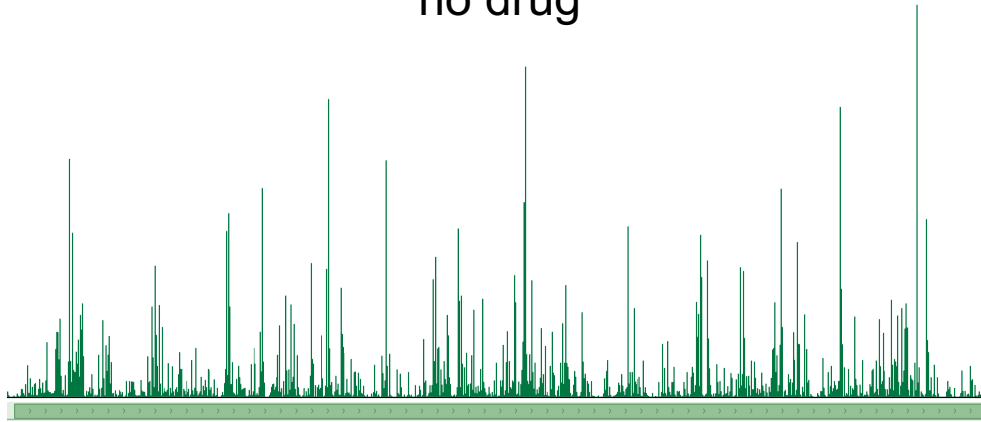


Orthosomycins bind at a unique ribosomal site

Evernimicin

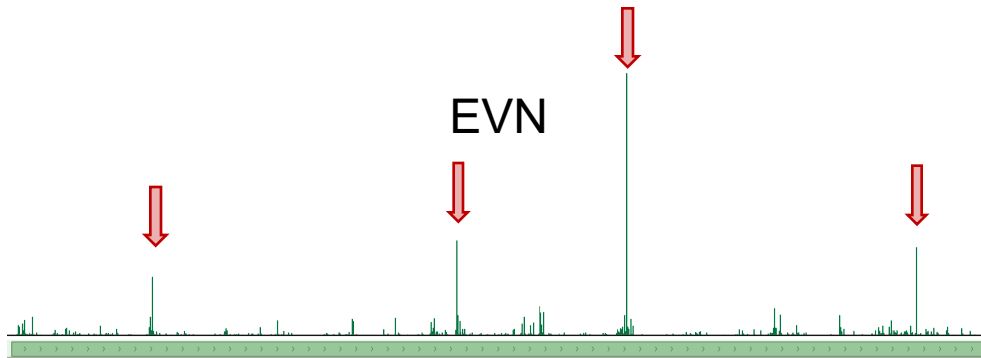


no drug



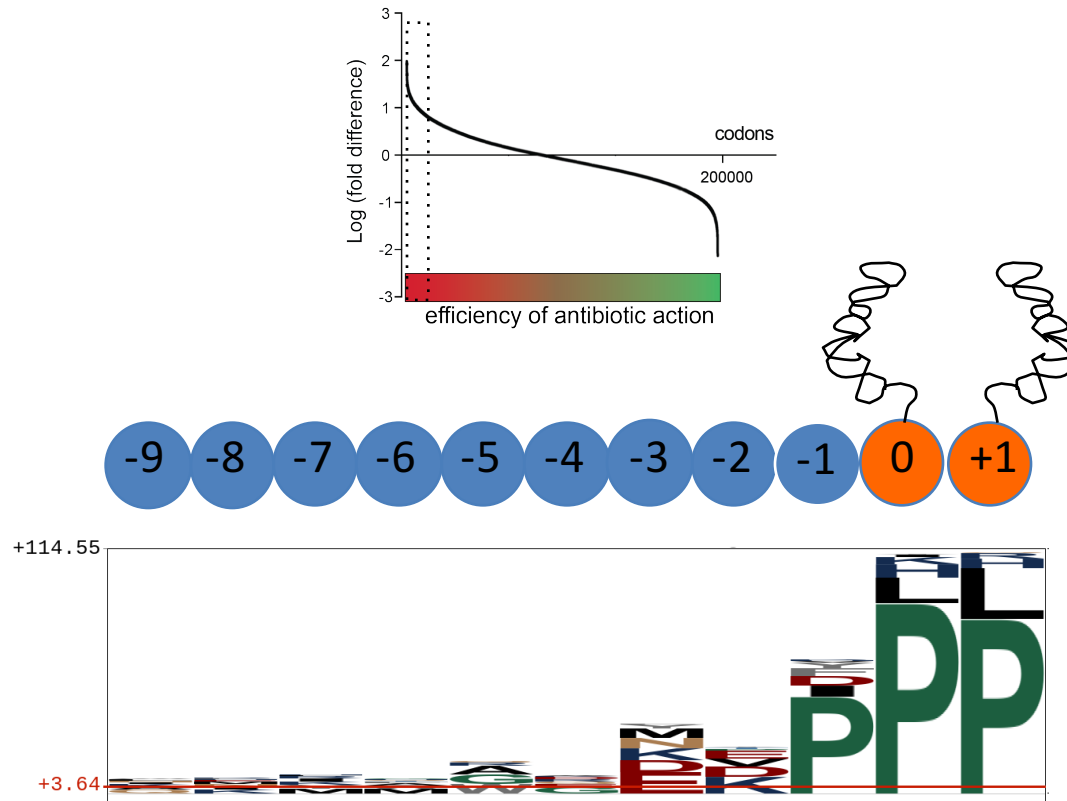
talB

EVN

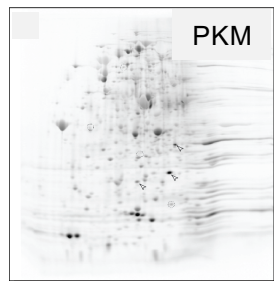
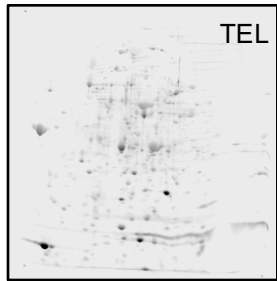
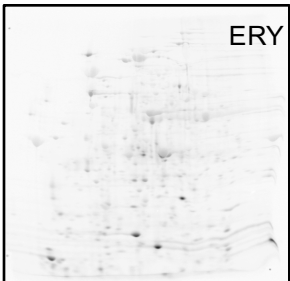
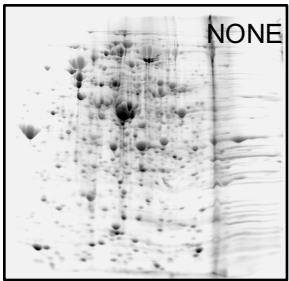
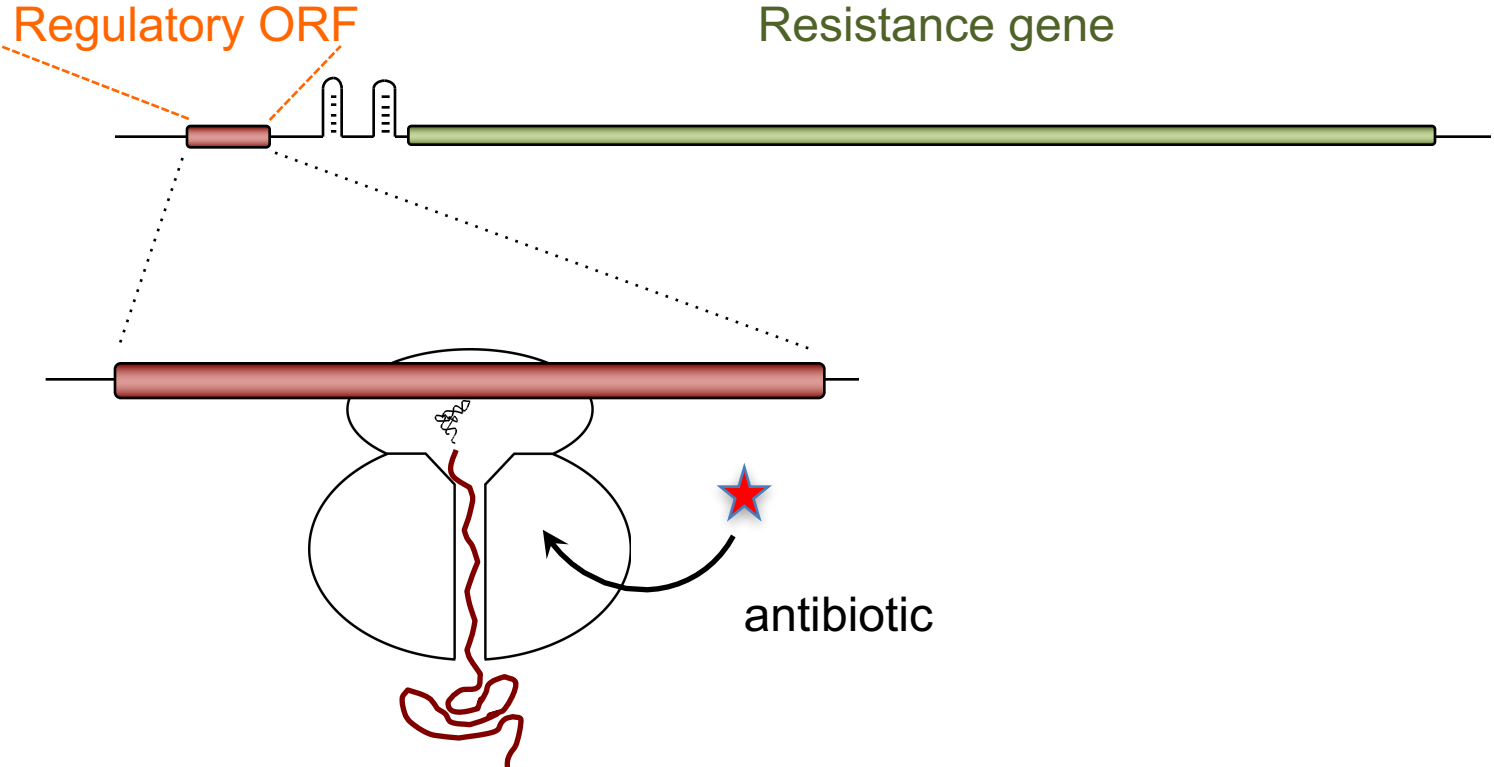


Limited number of sites of drug-specific arrest

Translation of the proline-rich sequences is preferentially inhibited by orsosomycins



Specificity of the antibiotic action is in the heart of the regulations of inducible resistance genes



- Many ribosomal antibiotics act in a context- and protein-specific manner.
- Regulation of resistance mechanisms often relies on context specificity of antibiotic action.
- Understanding the principles of context specificity is critical for the knowledge-based new antibiotic discovery and drug development



Sezen Meydan
Haripriya Ramu
Maxim Svetlov

Priya
Sothiselvam

Cedric
Orelle

Nora Vazquez-Laslop

Collaborations:

UCSF
Jonathan Weissman,
Eugene Oh

Leopold-Franzens University
Ronald Micura
Sandro Neuner
Lukas Rigger

Stanford University
Jody Puglisi
Choi Junhong

University of Illinois
Yury Polikanov

Mashal
Almutairi

Pulkit
Gupta

James
Marks

Dorota Vrinda Krishna Teresa
Klepacki Gupta Kannan Szal

Tanja
Florin

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