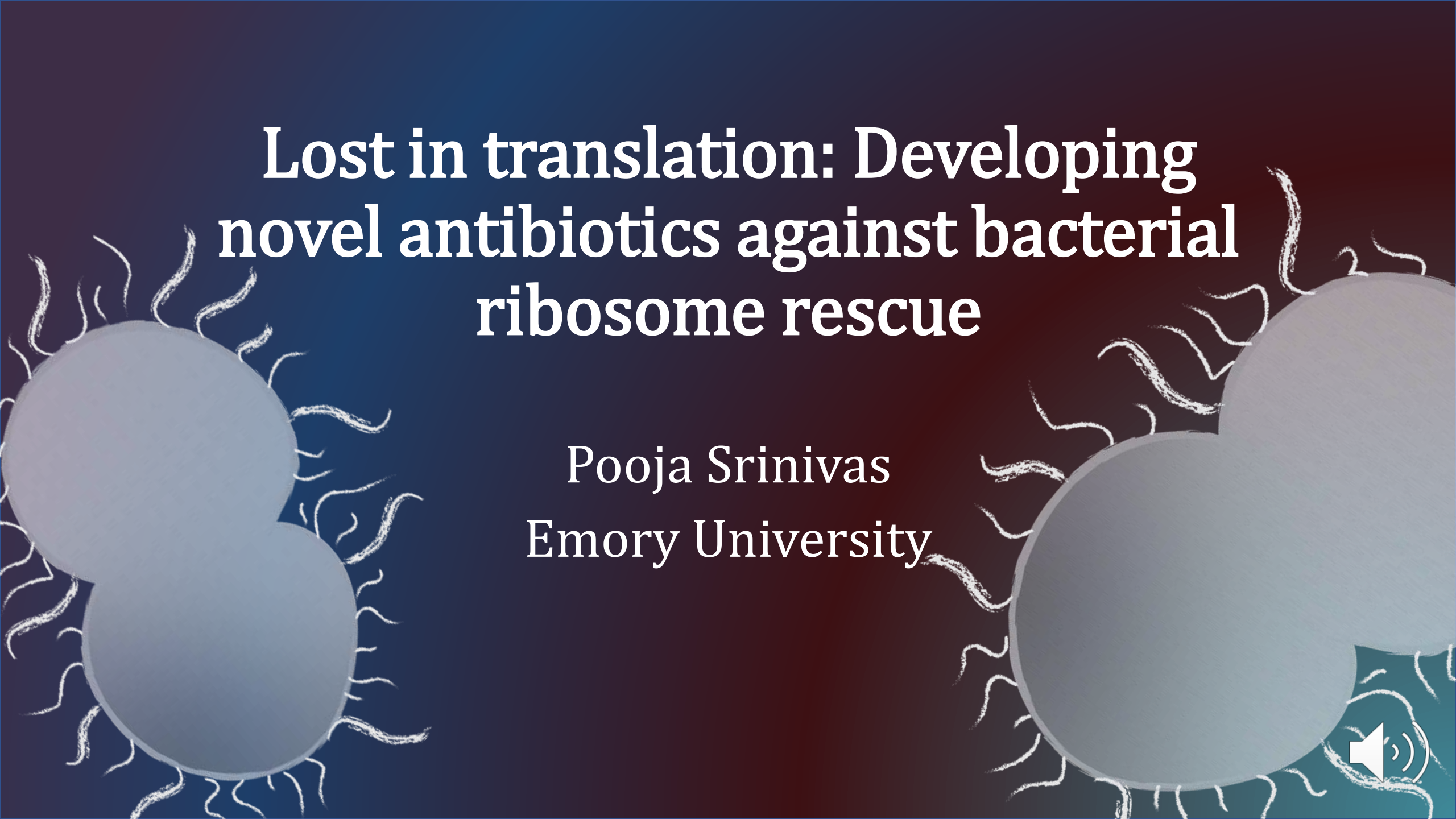
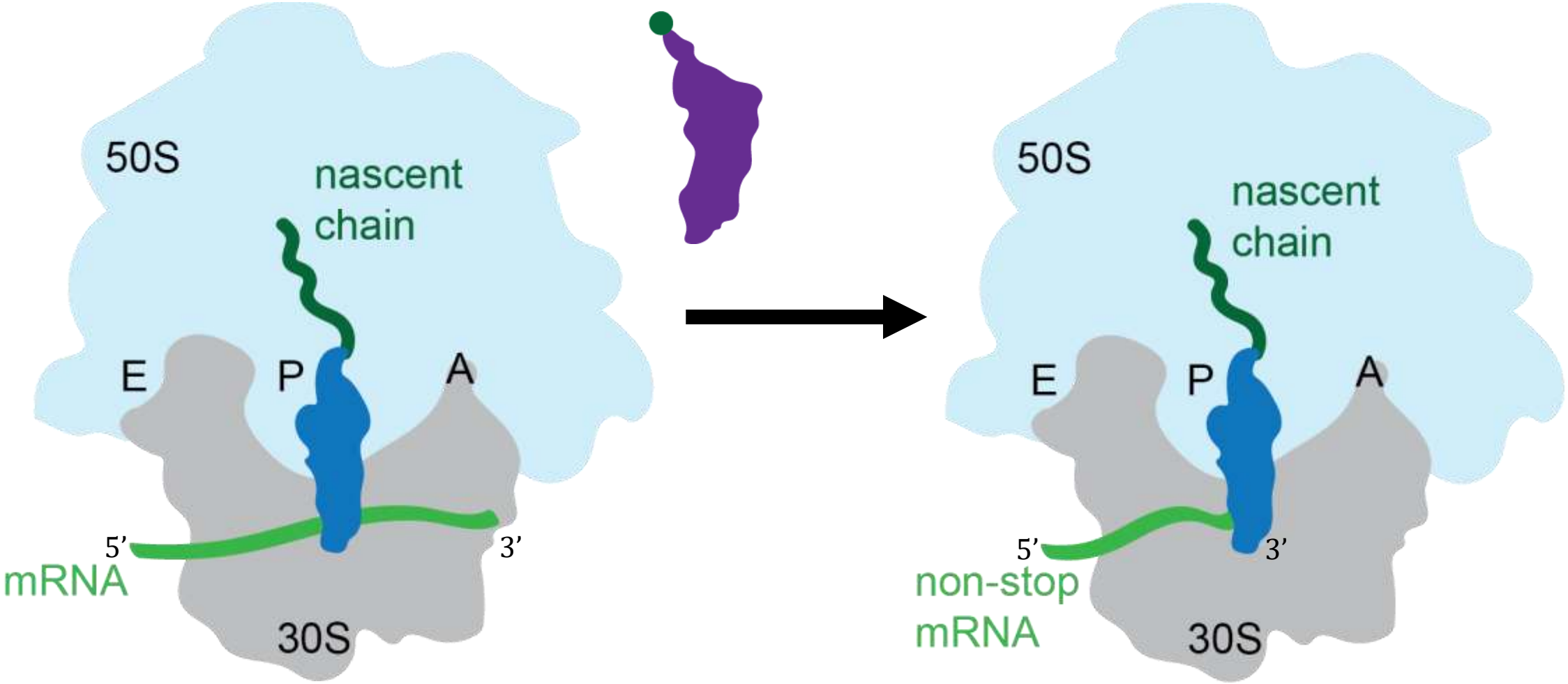
Lost in translation: Developing novel antibiotics against bacterial ribosome rescue

Pooja Srinivas
Emory University





Translating 70S Ribosome

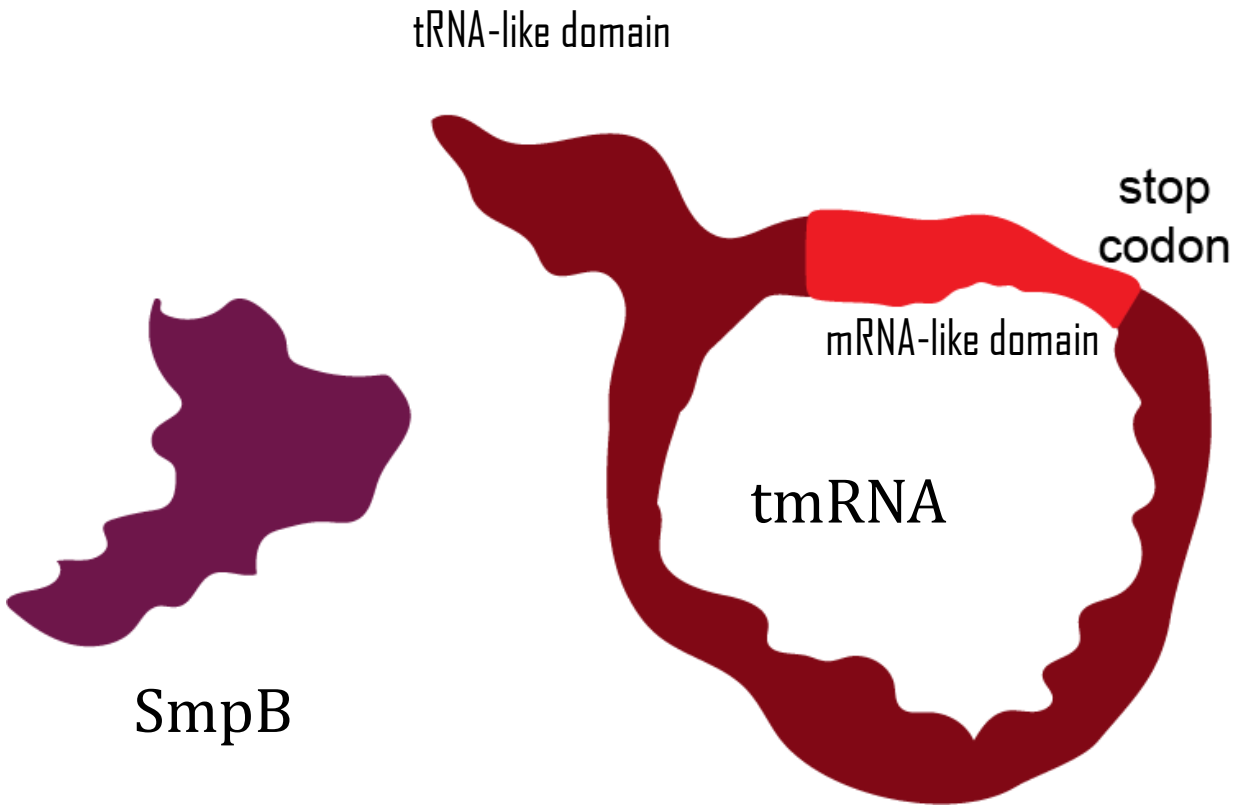
Non-stop 70S Ribosome

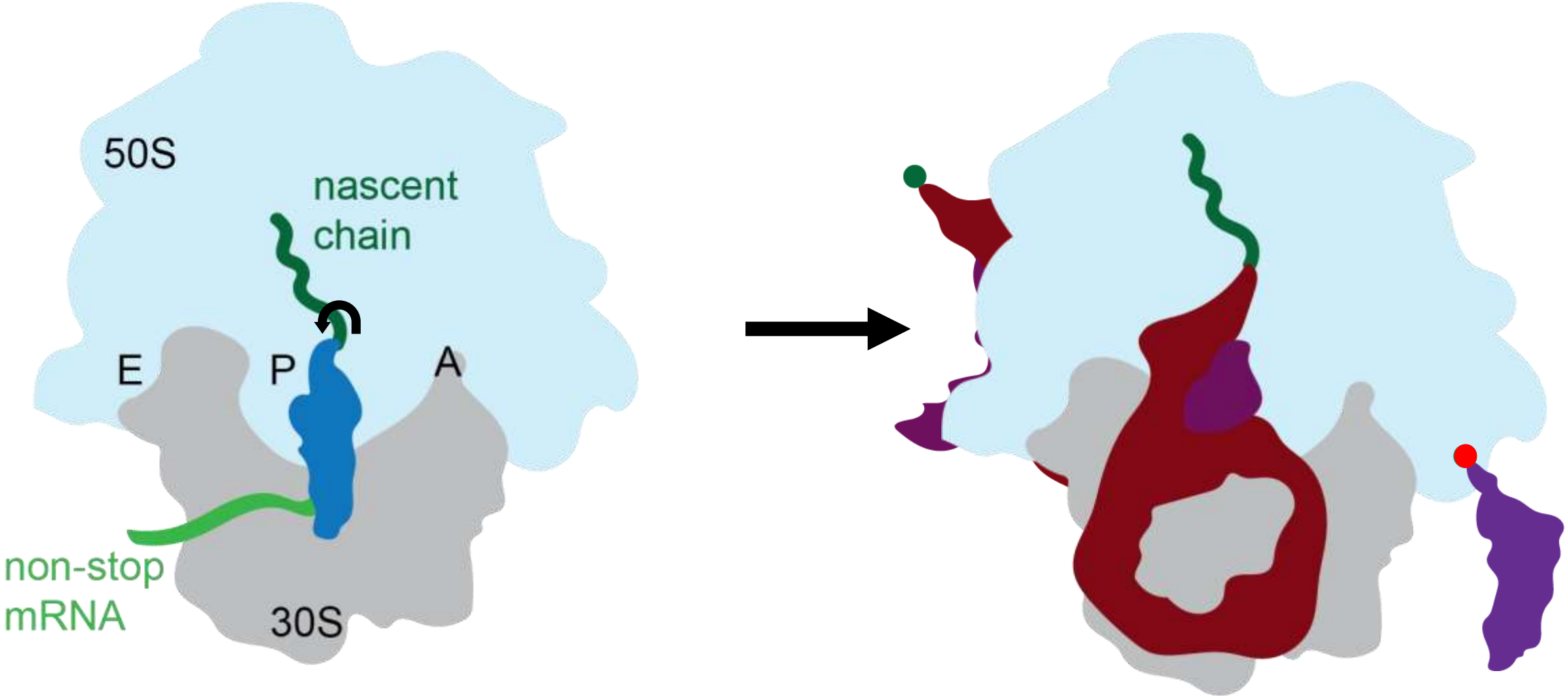
Buildup is lethal

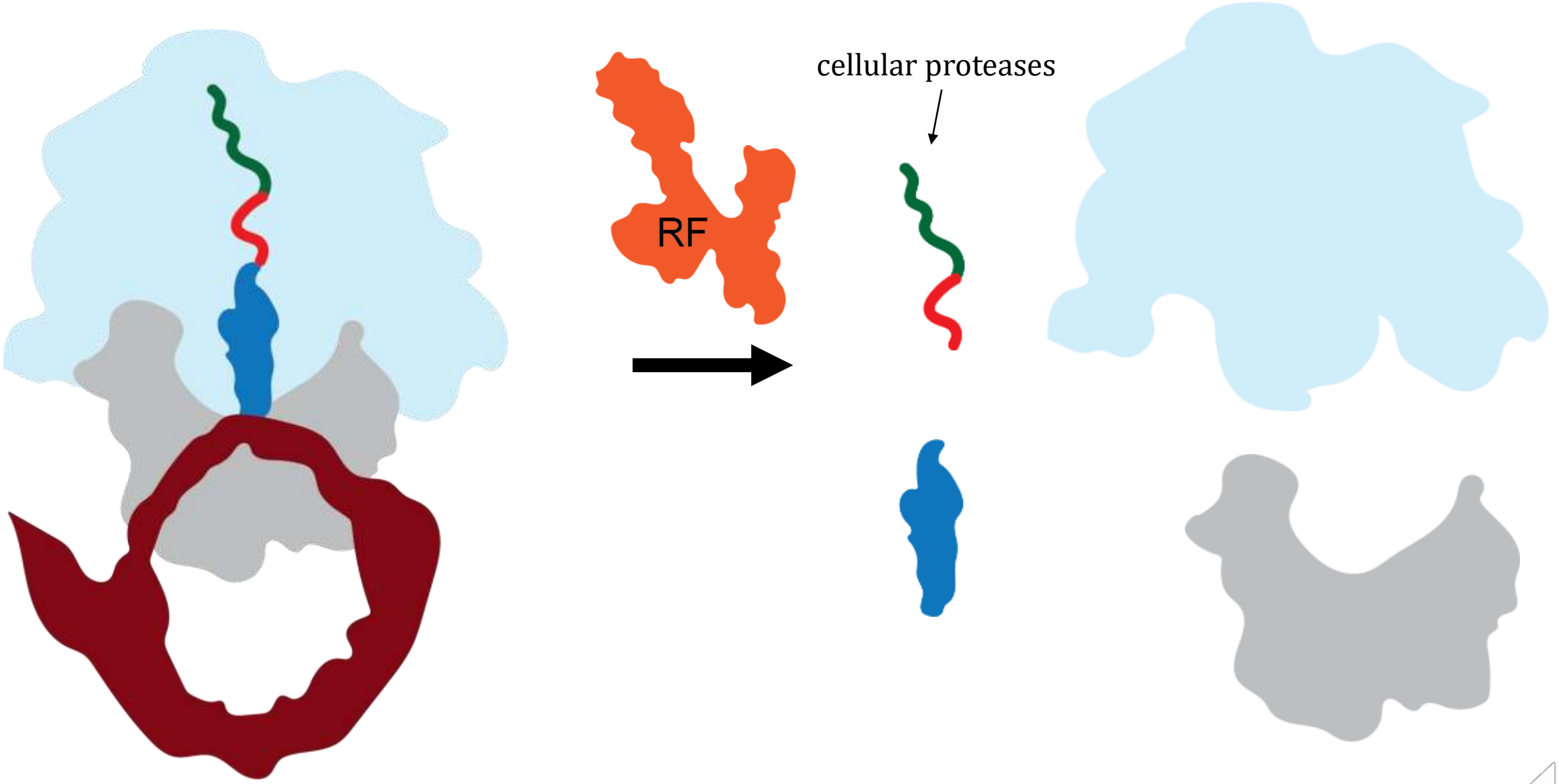
Trans-translation components

In bacteria:

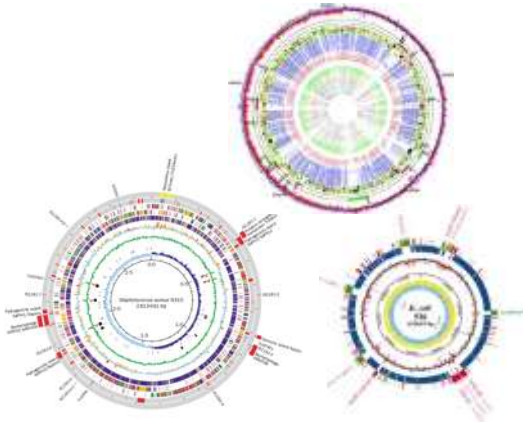
- SmpB: small protein B
- tmRNA: transfer-messenger RNA



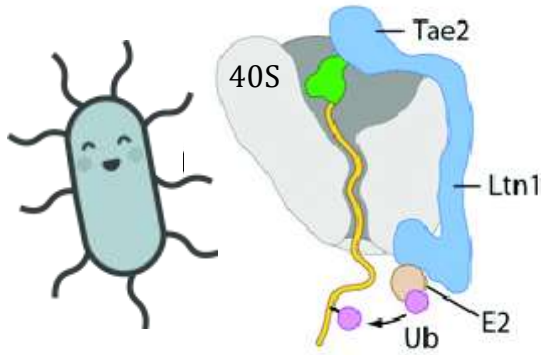

50S
nascent
chain
E
P
A
non-stop
mRNA
30S


RF
cellular proteases

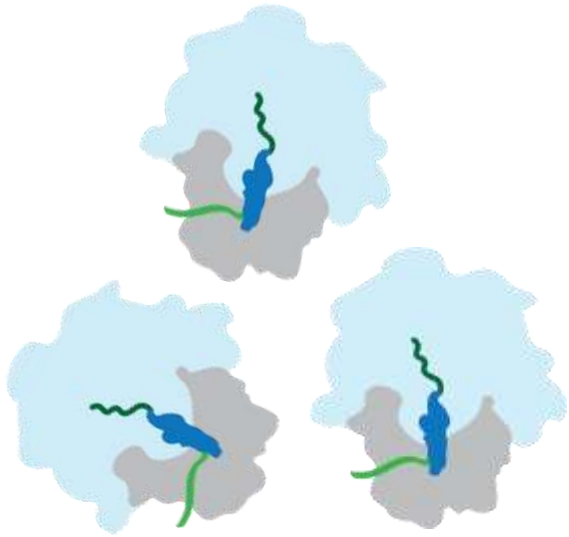
trans-translation as an antibacterial target



All bacterial genomes



Bacteria specific



High frequency

Essential

trans-translation inhibition increases non-stop complexes, leading to bacterial death

Trans-translation as an antibacterial target

Table 1. Antibacterial activity of small molecule inhibitors

	S. flexneri		*B. anthracis*		*M. smegmatis*	
Inhibitor	MIC*	MBC†	MIC	MBC	MIC	MBC
KKL-35	6.0 (0)	S‡	3.0 (2.6)	S	1.3 (0.4)	1.3 (0.4)
KKL-52	50 (0)	S	1.5 (0)	6.0 (0)	100 (0)	100 (0)
KKL-10	7.8 (5.5)	S	2.3 (0.3)	7.5 (6.4)	1.5 (0)	1.5 (0)
KKL-22	19 (11)	S	10 (3.5)	25 (0)	5.0 (1.7)	5.0 (1.7)
KKL-55	13 (9.0)	S	25 (0)	50 (0)	25 (0)	25 (0)

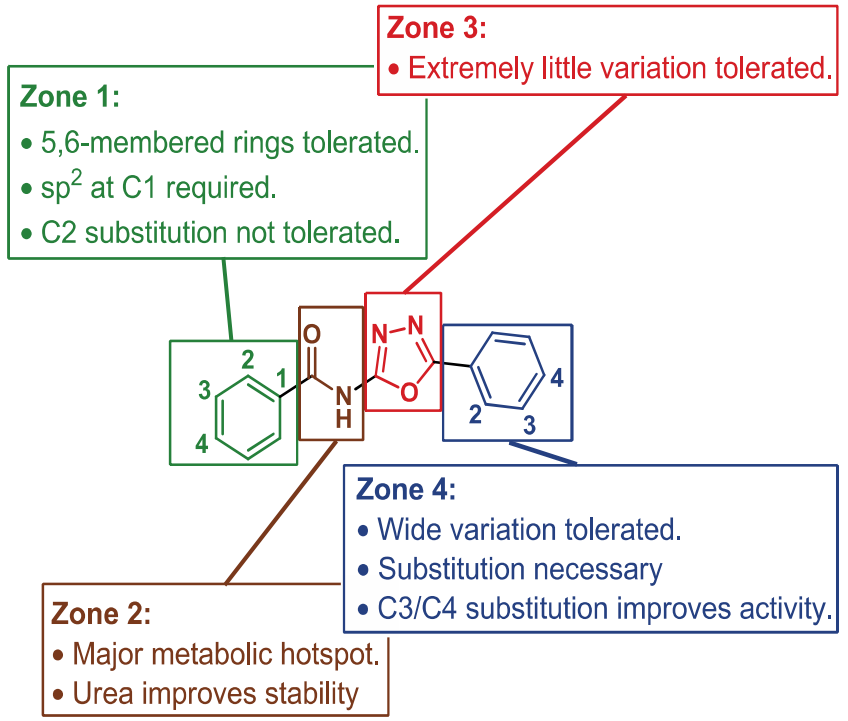
ND, not determined.
*Mean (SD) values from at least three broth microdilution assays (μM).
†Mean (SD) values from at least three plating assays (μM).
‡Bacteriostatic at all tested concentrations.

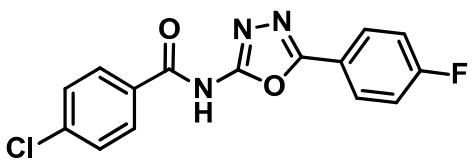
- ID small molecular inhibitors specific to *trans*-translation
- Broad-spectrum, bacteriostatic and bactericidal activity

Ramadoss, N., *et. al.* (2013) *PNAS*

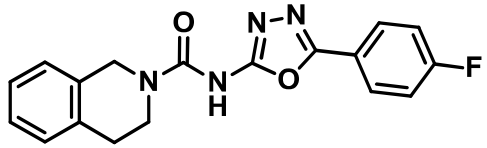
MBX-4132

- Synthesis to improve pharmacokinetics
  - Oral bioavailability/permeability
  - Metabolic stability
  - Maintain potency (MIC)
- MIC (*N. gonorrhea*): 0.18 ng/mL





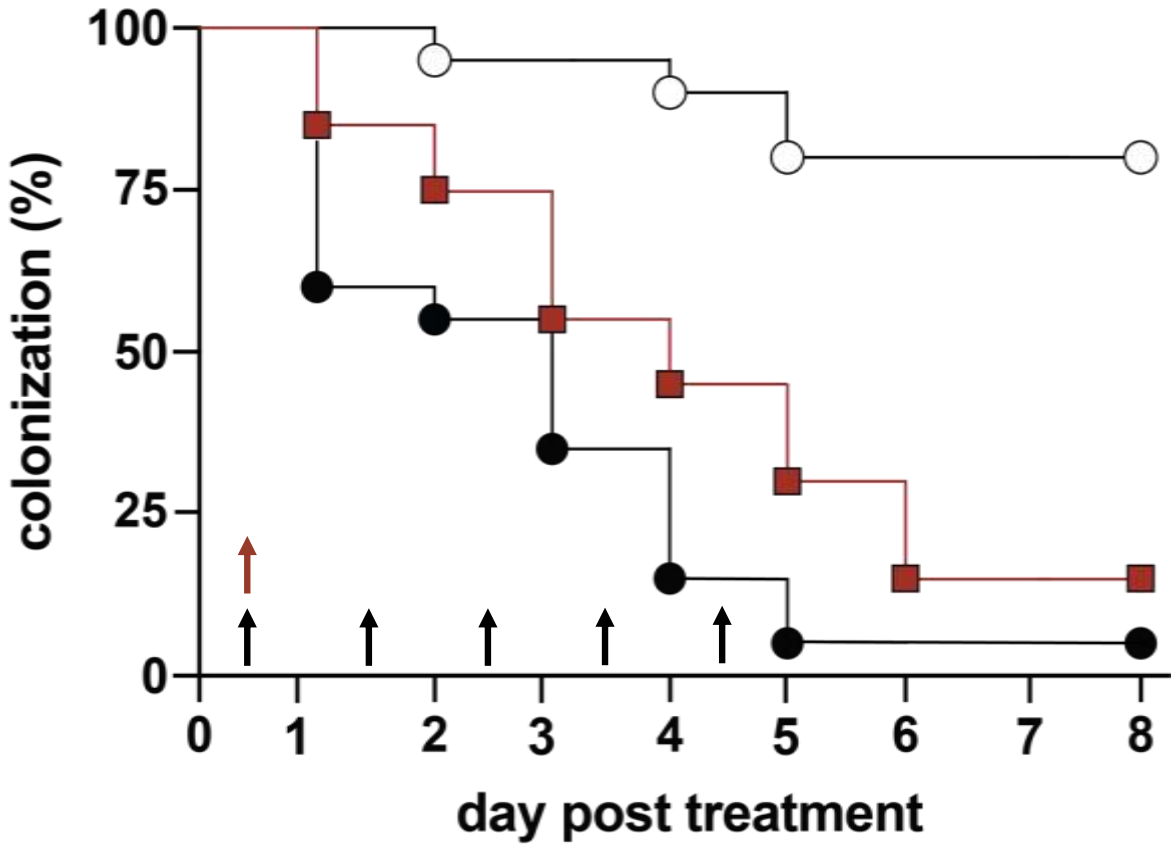
KKL-35



MBX-4132

Aron, *et. al.* (2021) *Nat Comm*

MBX-4132 clears MDR *N. gonorrhoeae* lower genital tract infection



MBX-4132

gentamicin

vehicle

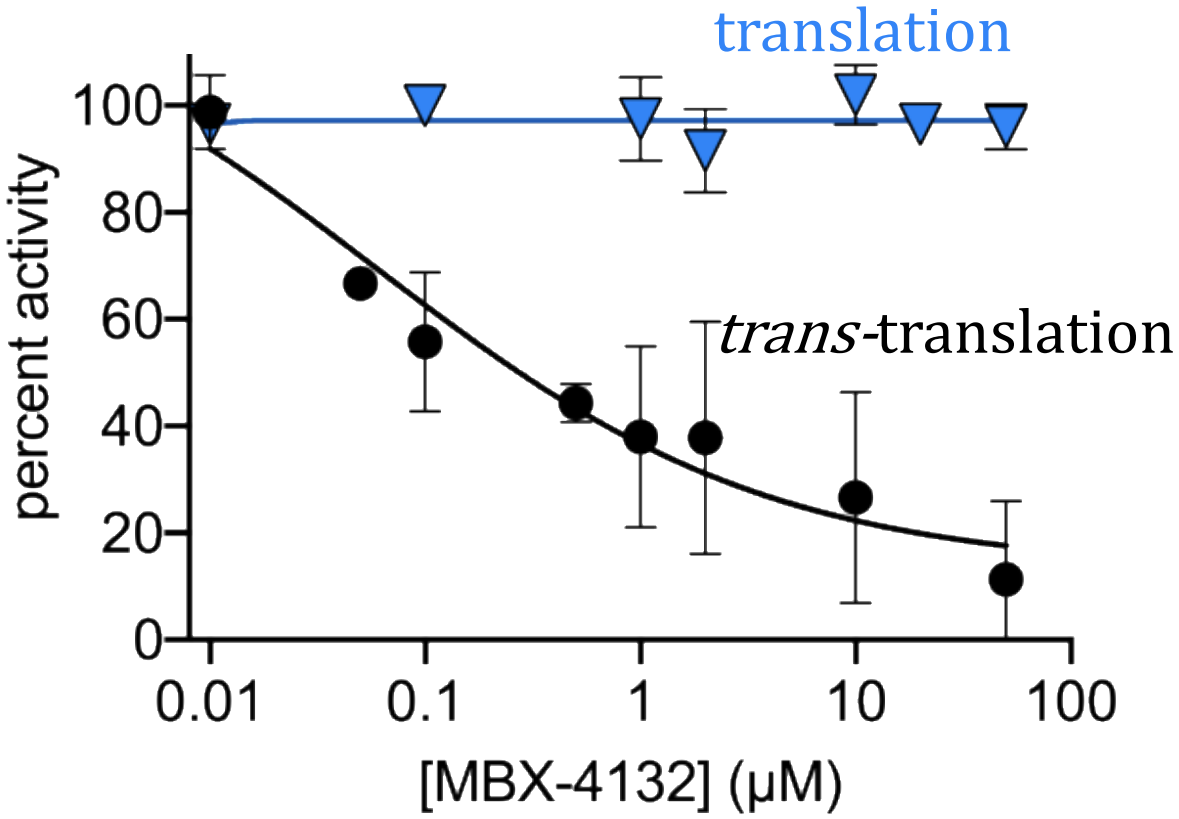
- n = 20 – 21 mice/group
- 10 mg/kg MBX-4132 (oral)
- 48 mg/kg gentamicin (IP)

Aron, *et. al.* (2021) *Nat Comm*

Determining a mechanism of action for MBX-4132

MBX-4132 specifically inhibits *trans-translation*

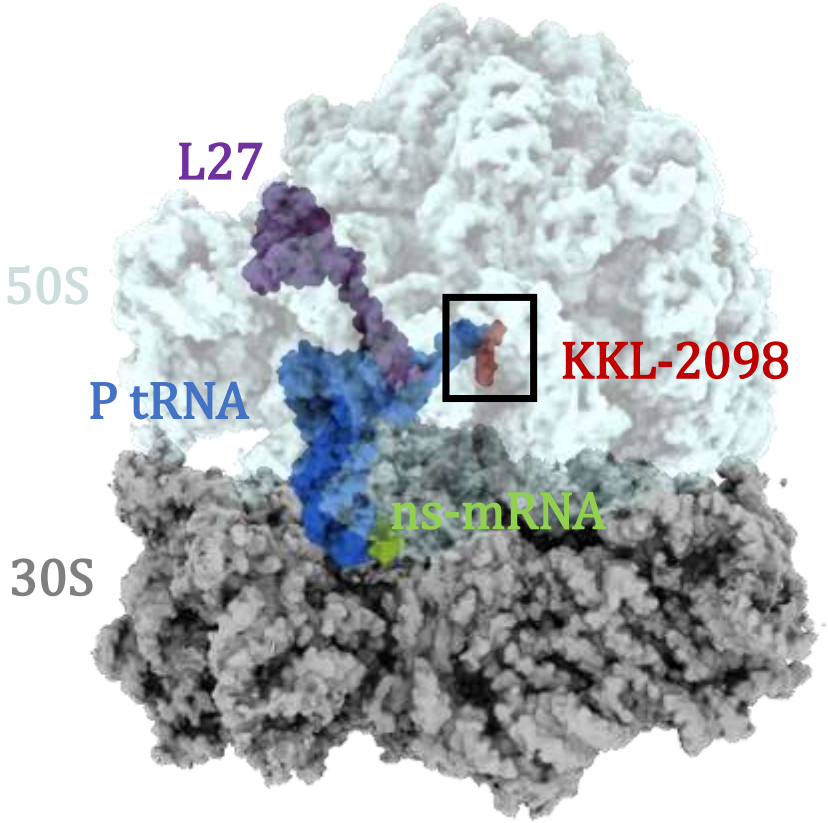
- *in vitro* translation
- Black: truncated nano-luciferase mRNA, tmRNA with end of transcript
  - *Trans*-translation **decreases**
- Blue: fl nano-luciferase mRNA
  - Normal translation **unaffected**

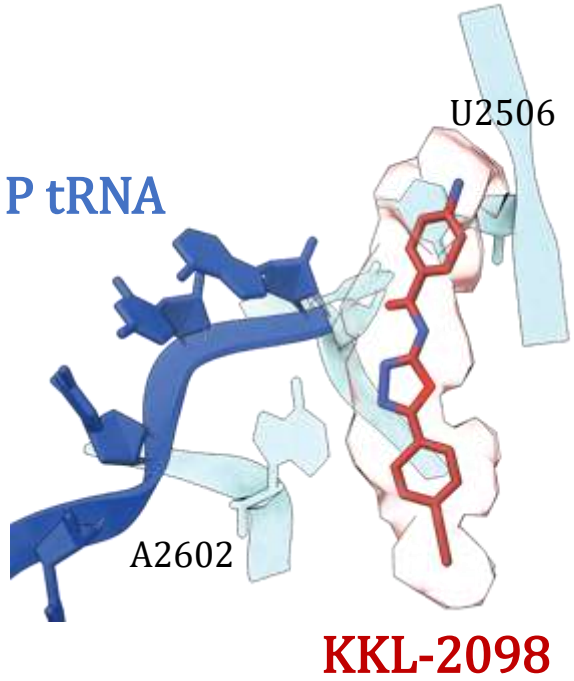


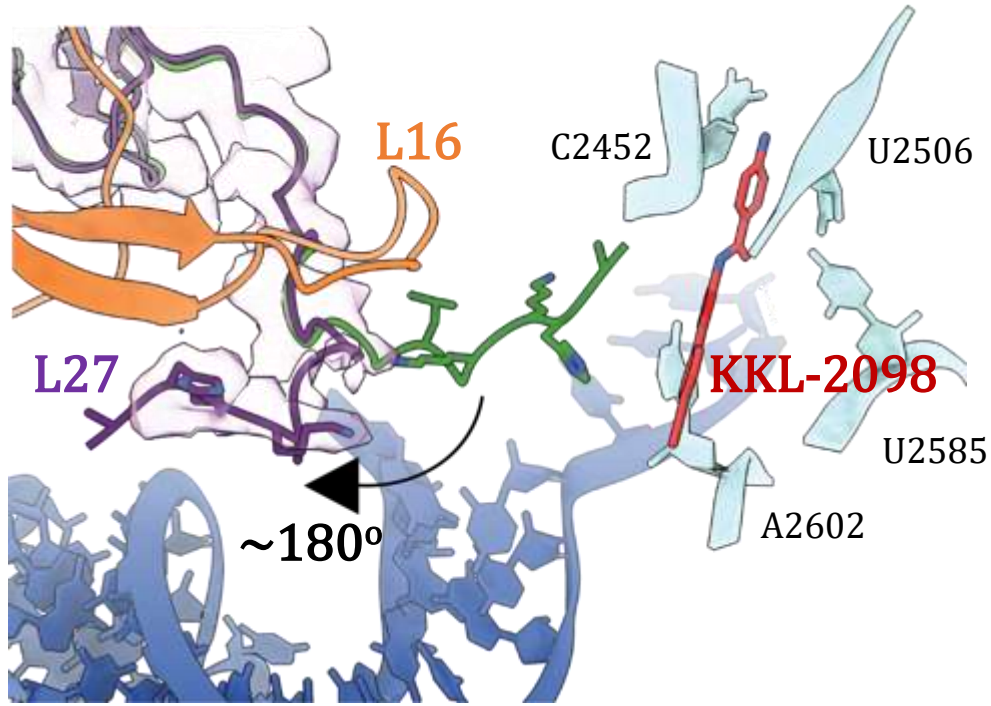
n = 3, mean ± SD

Aron, *et. al.* (2021) *Nat Comm*

3.1 Å cryo-EM structure reveals KKL binding site

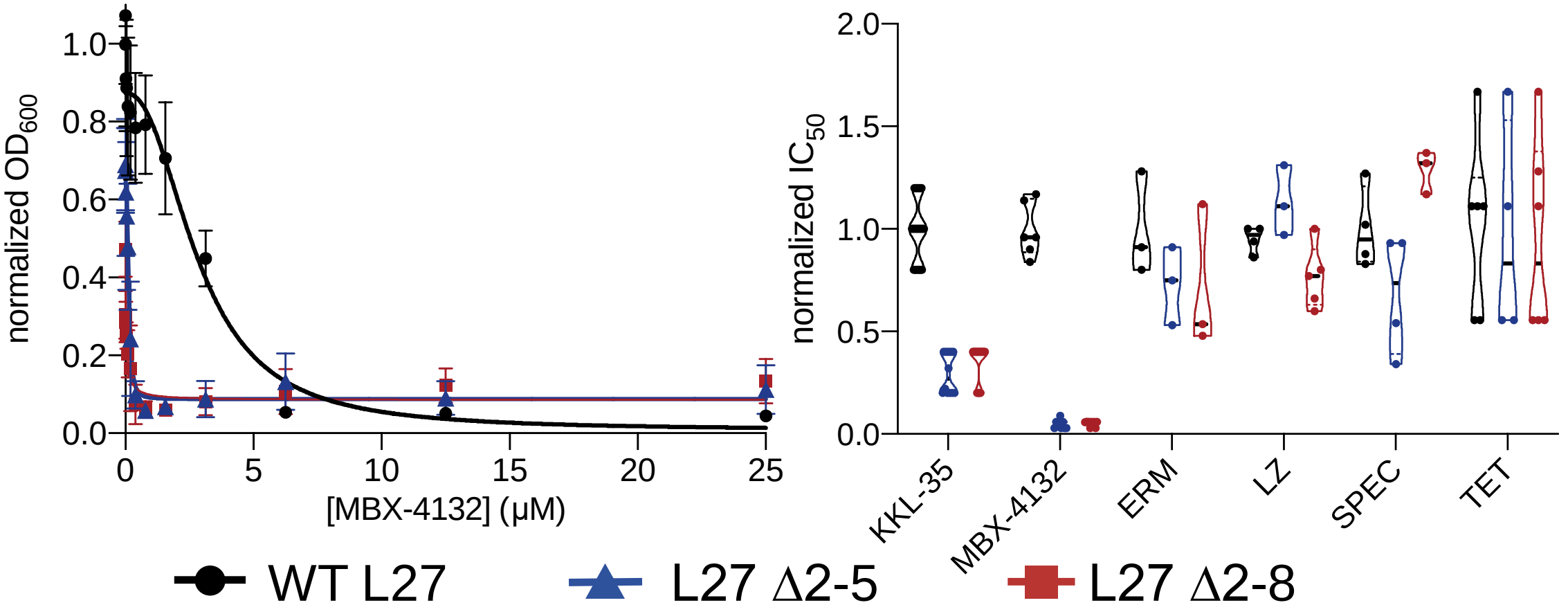






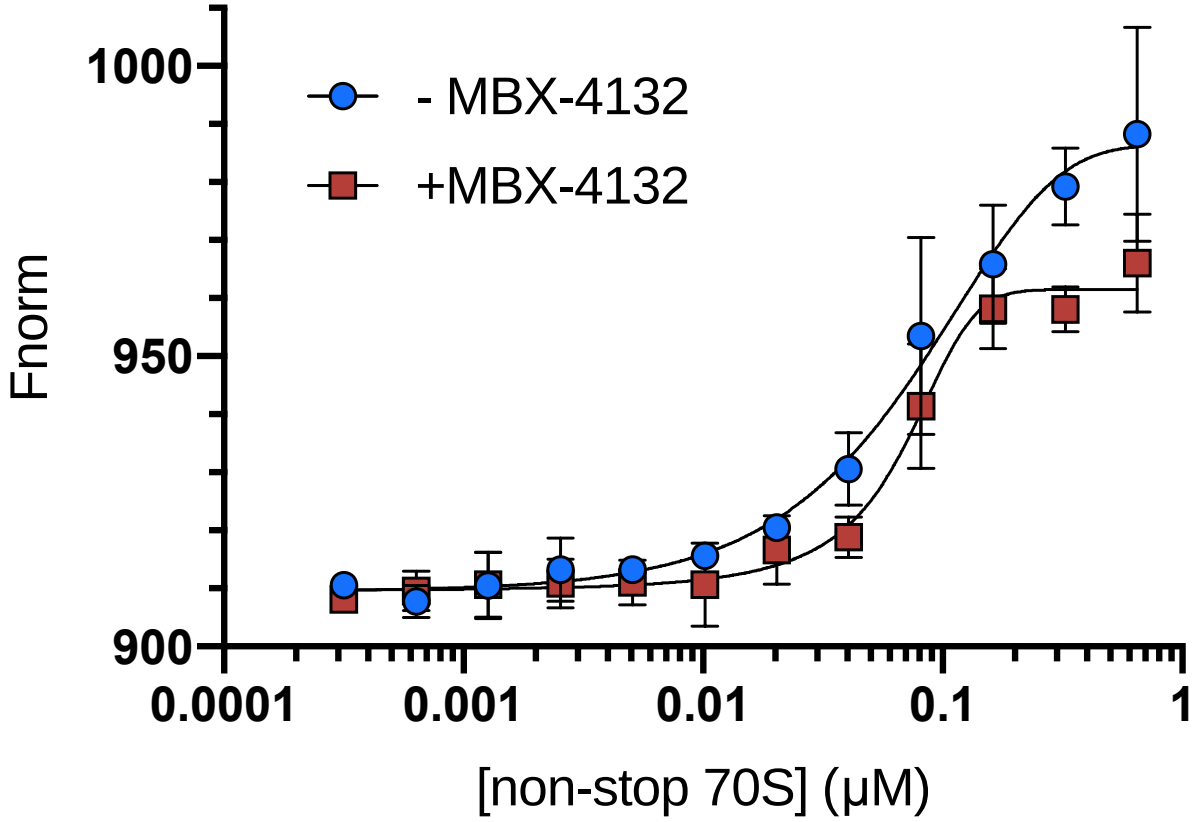
Aron, *et. al.* (2021) *Nat Comm*

L27 truncations increase the potency of *trans-translation* inhibitors



Aron, *et. al.* (2021) *Nat Comm*

MBX-4132 does not inhibit SmpB-tmRNA binding



Labeled SmpB-tmRNA added to titration series of ns-70S

- MBX-4132

K_{DAvg} = 70.5 nM ± 10.6 nM
n = 2

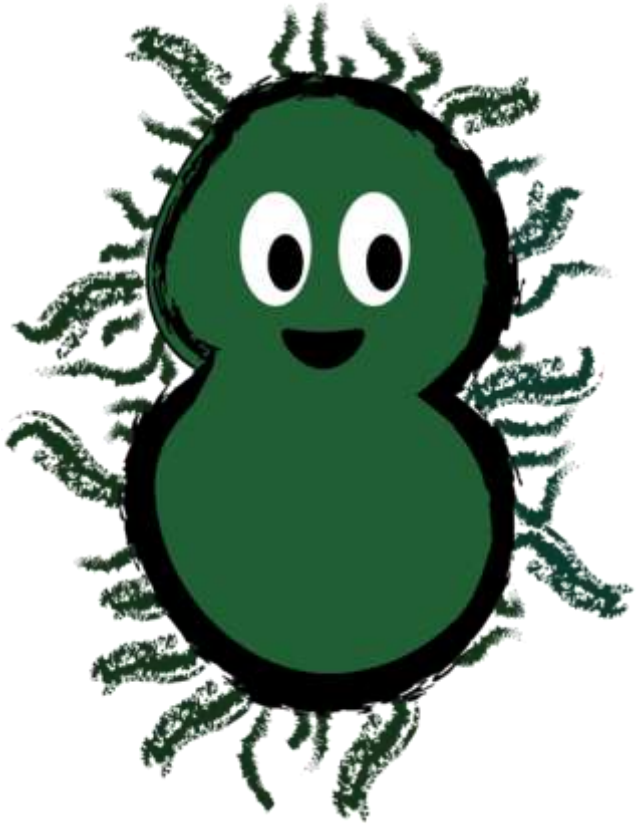
+ MBX-4132

K_{DAvg} = 80.3 nM ± 18.6 nM
n = 2

No difference in SmpB-tmRNA binding with MBX-4132

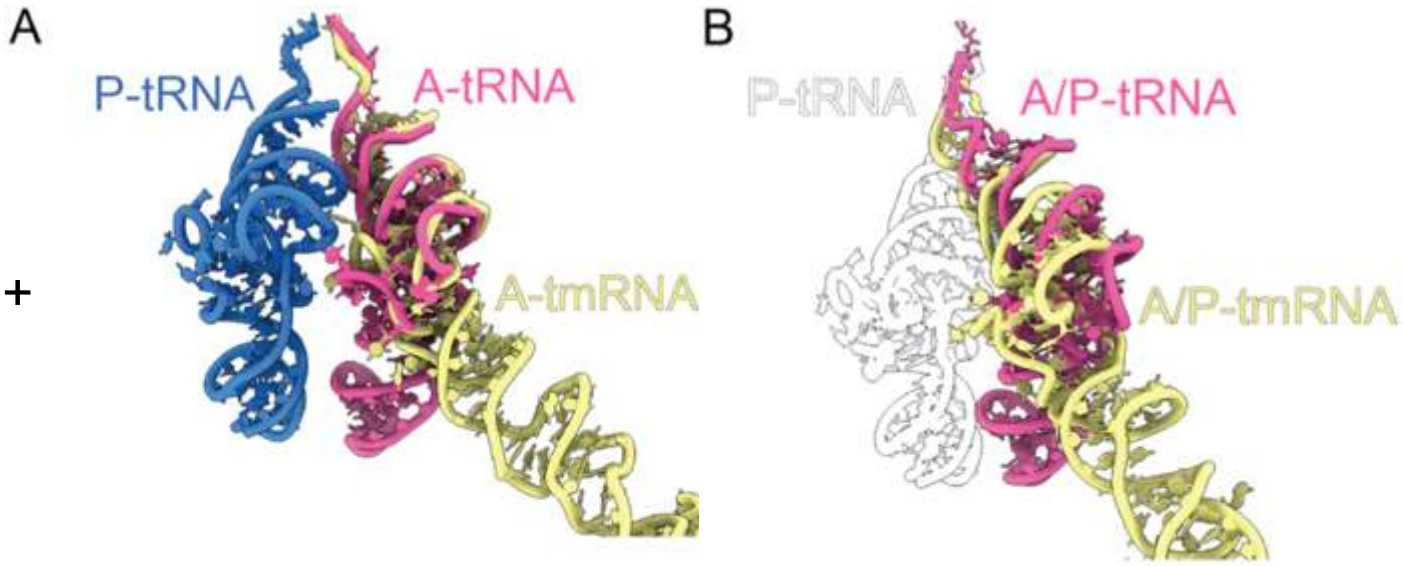
Conclusions

- MBX-4132 is a novel antibiotic to treat drug resistant *N. gonorrhea* infections in mice
- MBX-4132 specifically inhibits *trans*-translation in bacteria
  - Binds 70S near PTC
  - L27 conformation change
  - Prevents rescue, but has no effect on normal translation



Future Directions: Identify how *trans*-translation inhibitors work

1. Peptide bond formation
2. Translocation
- Cryo-EM structure of ns-70S + MBX-4132 + SmpB-tmRNA



Aron, *et. al.* (2021) *Nat Comm*

Acknowledgements

Dunham Lab

- Christine Dunham, PhD
- Eric Hoffer, PhD (former)
- Evy Kimbrough
- Lexie Kuzmishin Nagy, PhD
- Jacob Mattingly
- Ha An Nguyen, PhD (former)
- Ian Pavelich, PhD
- Eliseo Salas
- Julia Tanquary
- Tiffany Trieu

Keiler Lab

- Ken Keiler, PhD
- John Alumasa, PhD
- Mynthia Goss, PhD
- Divya Hosangadi

Stagg Lab

- Scott Stagg, PhD
- Atousa Mehrani

Funding

- NIH T32-GM008602
- NIH T32-AI106699
- NIH R01-GM093278
- NIH R01-GM065183

Microbiotix

- Zachary Aron, PhD
- Matthew Torha
- Jay Barbor
- Steven Cardinale, MS
- Michelle Butler, PhD
- Timothy Opperman, PhD
- Terry Bowlin, PhD

Others

- Ann Jerse, PhD, UHSU
- MSP Graduate Program
- Biochemistry Department

Committee

- Graeme Conn, PhD
- Renhao Li, PhD
- Eric Ortlund, PhD
- Bill Shafer, PhD

Questions?