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Molecular mechanisms of antibiotic resistance

The ribosome is essential for all living organisms, and the bacterial ribosome is an important antibiotic target. There are drugs that inhibit every step of bacterial protein synthesis, and bacteria have evolved a large variety of resistance mechanisms against these drugs[1].

We use structural biology methods in combination with biochemistry and biophysics to understand the detailed mechanism of resistance to translation-inhibiting antibiotics. This involves resistance enzymes that inactivate antibiotics[2,3], resistance enzymes that post-transcriptionally modify rRNA to sterically block the drug-binding site on the ribosome[4], and resistance proteins that in other ways allow protein synthesis to proceed in presence of a drug[5].

We currently have two project suggestions relating to molecular mechanisms of antibiotic resistance. Both projects can be adapted to the interest of the student.

A: Antibiotic resistance through methylation

Ribosomal RNA methyltransferases that mediate antibiotic resistance have to recognize and modify a specific rRNA position during synthesis and assembly of the ribosome. The aim of this project is to find out how such an enzyme recognizes the target in a partially assembled ribosome. The project will involve protein expression and purification, binding studies and single-particle cryo-EM.

B: Factor-mediated antibiotic resistance

FusB-like proteins mediate resistance against fusidic acid through an interaction with elongation factor G[5,6]. The aim of this project is to characterize a single-domain FusB homologue only containing the C-terminal domain (Fig. 1) encoded in an operon together with a transcriptional repressor and another potential resistance enzyme. We aim to figure out whether this single-domain FusB can bind to EF-G and mediate fusidic acid resistance. Potentially the project could also be extended to characterize the function of the full operon. The project will involve protein expression and purification, binding studies, crystallization and potentially X-ray crystallography.

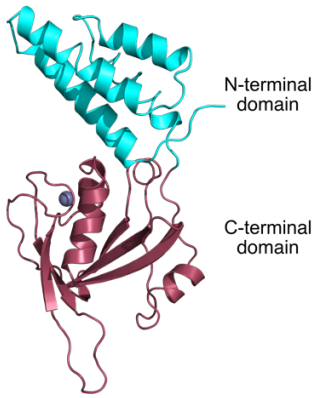

Figure 1. Structure of FusB from S. aureus (PDB: 4ADN)[5].

References

1. Wilson, D. N. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nat. Rev. Microbiol.* **12**, 35–48 (2014).
2. Stern, A. L. *et al.* Structural mechanism of the aminoglycoside (3")(9) adenyl transferase AadA from Salmonella enterica. *J. Biol. Chem.* **293**, 11481–11490 (2018).
3. Kanchugal P, S. & Selmer, M. Structural Recognition of Spectinomycin by Resistance Enzyme ANT(9) from Enterococcus faecalis. *Antimicrob. Agents Chemother.* **64**, e00371-20 (2020).
4. Stsiapanava, A. & Selmer, M. Crystal structure of ErmE - 23S rRNA methyltransferase in macrolide resistance. *Sci. Rep.* **9**, 14607 (2019).
5. Guo, X. *et al.* Structure and function of FusB: an elongation factor G-binding fusidic acid resistance protein active in ribosomal translocation and recycling. *Open Biol.* **2**, 1–12 (2012).
6. Tomlinson, J. H., Kalverda, A. P. & Calabrese, A. N. Fusidic acid resistance through changes in the dynamics of the drug target. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 25523–25531 (2020).