

Supplementary Information - High-throughput CryoEM methods, as exploited to support this two-pronged approach to antibiotic resistance, are ideal as it is possible to achieve sample preparation, data collection, and 3D reconstruction within one week. With sufficient microscope access, iterative rounds of development provide true, rapid structure-based drug design. With sufficient synthetic chemistry support, such an approach could inform and be applied to other antibiotics that currently have limited clinical value.

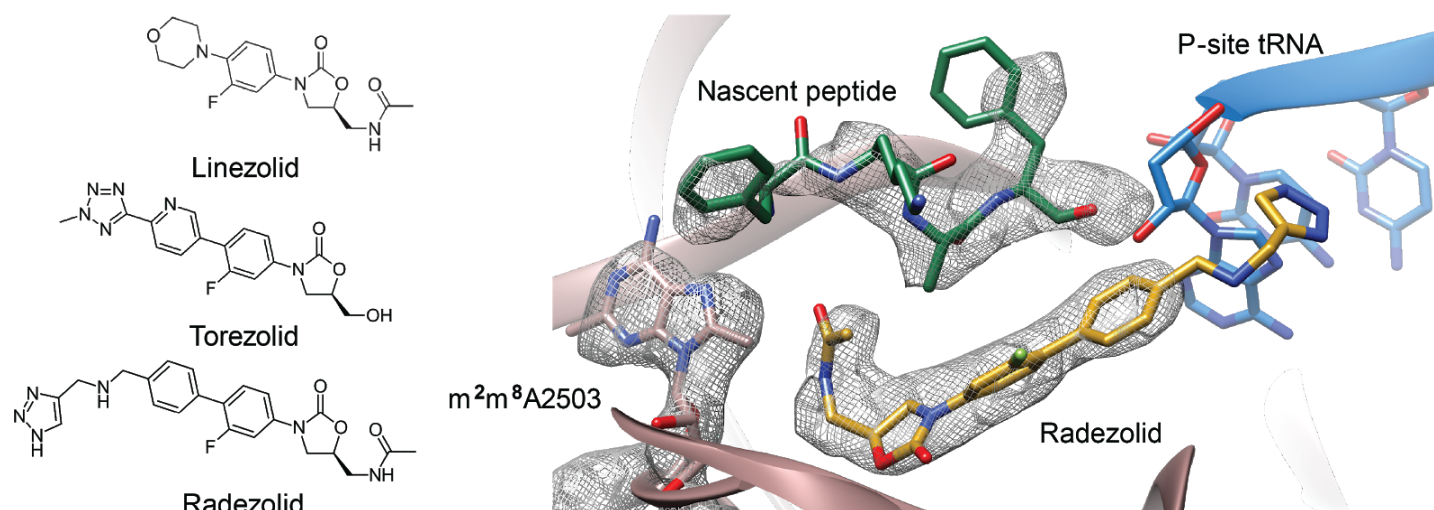


Figure 1: Examples of oxazolidinone antibiotics. At right, preliminary CryoEM structural information (2.51 Å reconstruction) revealing radezolid binding in a Cfr-methylated (m^2m^8 A2503) PTC. Improvements to the model and the parameterization of the radezolid are underway.

Preliminary Data and Sample Verification - Several preliminary oxazolidinone-bound and streptogramin-bound ribosomes have been characterized, including 50S, 70S, methylated 70S, and complexes stalled during translation. We are now on subsequent generations of designed inhibitors.

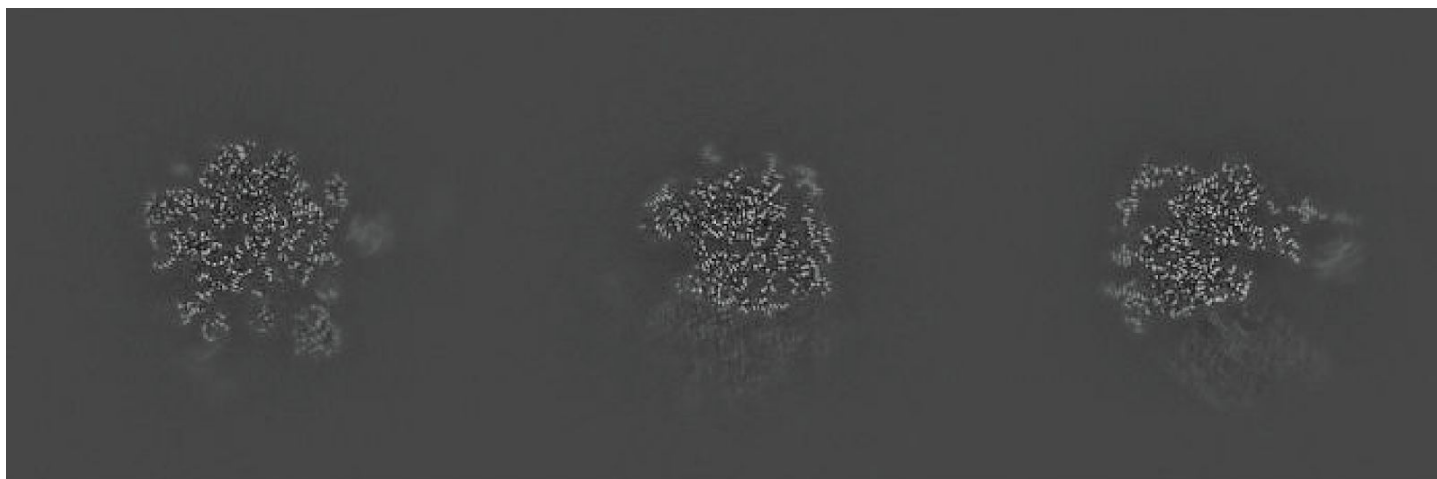


Figure 2: Preliminary data - Orthogonal bisections of an inhibitor-bound 50S ribosome. Currently at 1.9 Å in cisTEM, density for the streptogramin analog was strong. Very slight occupancy of the small subunit observed, will be addressed with masking. Two days of collection on a Titan Krios equipped with a GIF and a Gatan K3.

Proposed Experiments - The biggest obstacle in this project is microscope access. The sample preparation and biochemistry have been worked out in detail, but insufficient access to Titan Krios instruments that can produce high quality data is the major hindrance. To further the aims of this project, rapid collection of streptogramin-bound ribosomes or high-resolution maps which can resolve the A2503 methylation clearly in the presence of multiple oxazolidinone inhibitors are needed. We have several samples of our next-generation inhibitors bound to ribosomes, frozen, screened, and ready for data collection.