# Structural Studies on the Inhibition of Protein-RNA Drug Targets & Applications to the Development of Novel Therapeutics

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**Abstract:**
We study the molecular mechanisms of protein-RNA complexes and their applications for the development of novel inhibitors. We are interested in AminoAcyl-tRNA Synthetases (AARS), a family of proteins responsible for attaching the correct amino acid to the cognate tRNA, a process called aminoacylation; and preventing mischarging of tRNAs to non-correct amino acids (editing). AARSs are interesting therapeutic targets and housekeeping proteins that: *i)* play a fundamental role for protein translation, a critical process during fast growing conditions of microbes; and *ii)* present differences between microbes and humans that can be exploited for drug discovery [1]. We focus on Leucyl-tRNA synthetase (LeuRS), which charges leucine to the cognate tRNA and prevents mischarging to related amino acids. We have solved structures of LeuRS in different functional states: 1) the aminoacylation state, where the 3'-tRNA is in the synthetic site ready to accept the activated amino acid (leu-AMP); 2) the post-transfer editing state, where the mischarged 3'-tRNA is bound into the editing site for proof-reading. Our structures provided unprecedented insight into the structural dynamics and the translocation of the 3'-tRNA between the synthetic and the editing sites, separated > 30 Å [2,3] . Using this mechanistic insight, in collaboration with pharmaceutical companies, we contributed to the development of novel antibiotics, named benzoxaboroles, targeting LeuRS. Our structures of LeuRS–tRNA bound to the antibiotic unveiled a novel inhibition mechanism whereby the tRNA gets trapped at the editing site and effectively inhibits protein synthesis (**Fig. 1**).
Using a structure-based drug design approach we brought the potency of initial hits from micro- to nano-molar and improved selectivity, allowing their progression into clinical trials (phase II) for the treatment of complicated infections by Gram-negative bacteria. Exploiting this mechanism of action, we also discovered novel antiinfectives against apicomplexan parasites. Another area of our research is the targeting of other protein-RNA complexes such as Cleavage and Polyadenylation Specificity Factors (CPSF). We discovered a novel benzoxaborole inhibitor of eukaryotic parasites that targets CPSF3 [4]. In eukaryotes, 3' pre-mRNA processing mainly occurs via the concerted action of a multiprotein complex that cleaves the 3'-end of unmatured mRNAs before the addition of the poly-A tail and their export to the cytoplasm for protein translation. CPSF3 has a key function within this complex as it is the endonuclease cleaving 3' pre-mRNAs. I will discuss the details of this new protein-RNA inhibition mechanism, which relies on the capture of the metal-dependent mRNAse activity of CPSF3 [5].

**References:**

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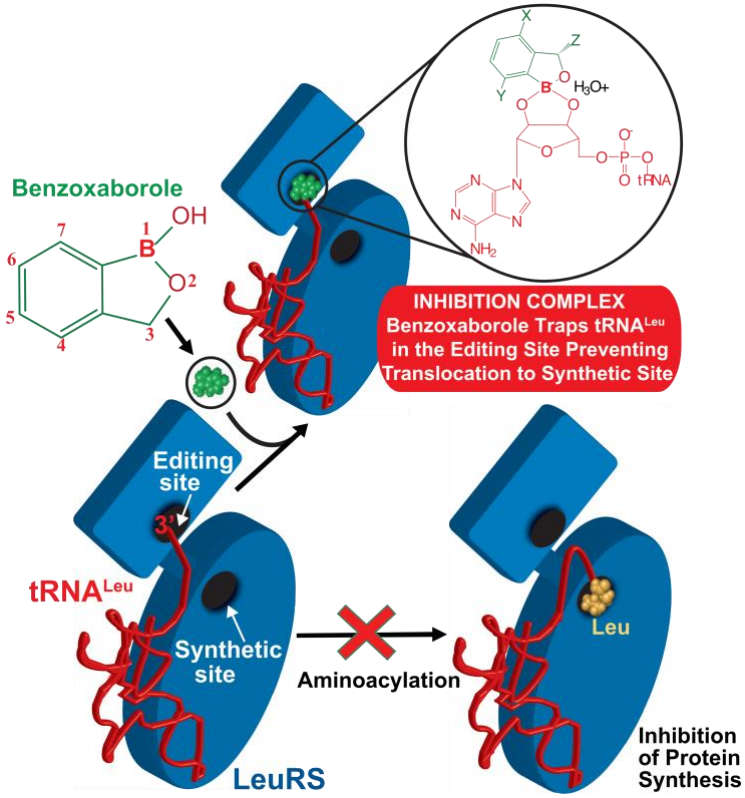


***Fig. 1.*** *Benzoxaborole mediated tRNA-trapping inhibition mechanism of LeuRS.*