Discovery of Small-Molecule Inhibitors of the Bacterial Ribonuclease P

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Abstract

We screened two small-molecule libraries by fluorescence spectroscopy, biolayer interferometry and X-ray crystallography against the P protein subunit of bacterial ribonuclease P as means to discover novel antibacterial compounds. We detected two crystallographic hits: 2-MBX and FTU, which both bind in a site relevant for the function of the RNase P holoenzyme. Finally, we detected the novel inhibitor purpurin which binds to the P protein in the same binding site as that of the pre-tRNA 5'-leader sequence.

Introduction

Ribonuclease P (RNase P) is a ribozyme (1) that catalyses the hydrolysis of the 5' end of all tRNA precursors across all domains of life, producing a mature tRNA and a 5' leader sequence. According to the crystal structure of bacterial RNase P (2), it is formed by a big RNA subunit (110 kDa) with a universally-conserved catalytic site and by a small protein subunit (P protein, 14.3 kDa) (Fig. 1), which does not present structural or sequence homology with its archaeal and eukaryal counterparts. Because of these reasons, we consider the P protein subunit as an important target for the development of novel wide-spectrum antibiotics (3).

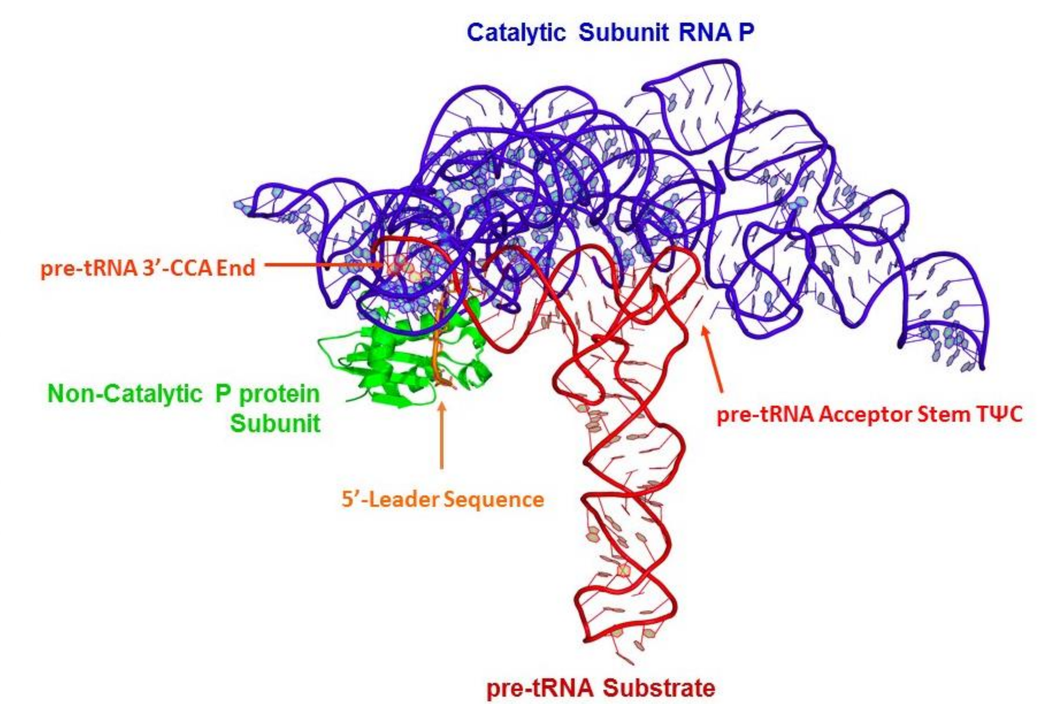


Figure 1. Crystal structure of bacterial RNase P ternary complex from *T. maritima*.

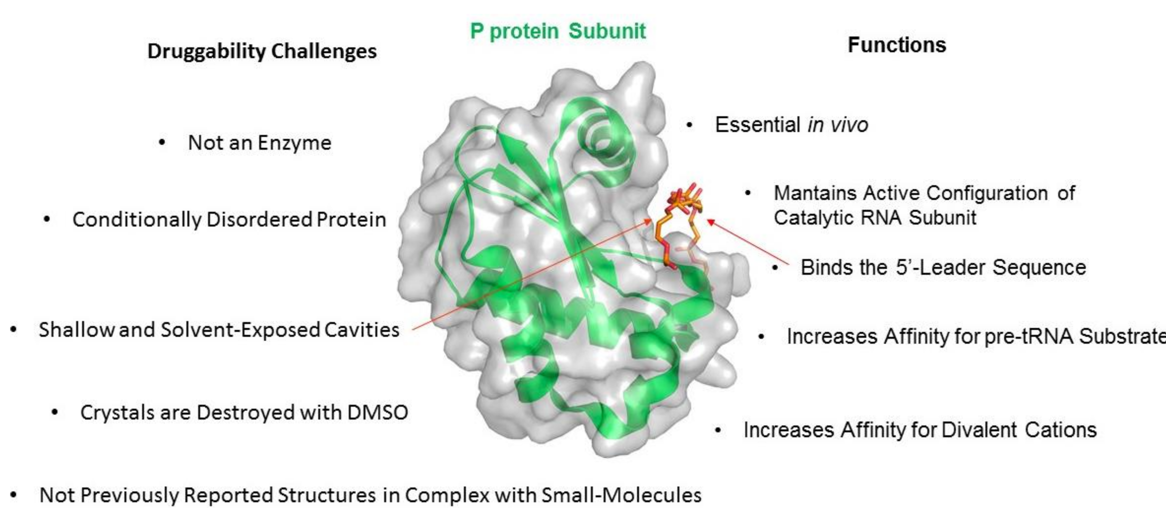


Figure 2. Functions of the RNase P protein subunit.

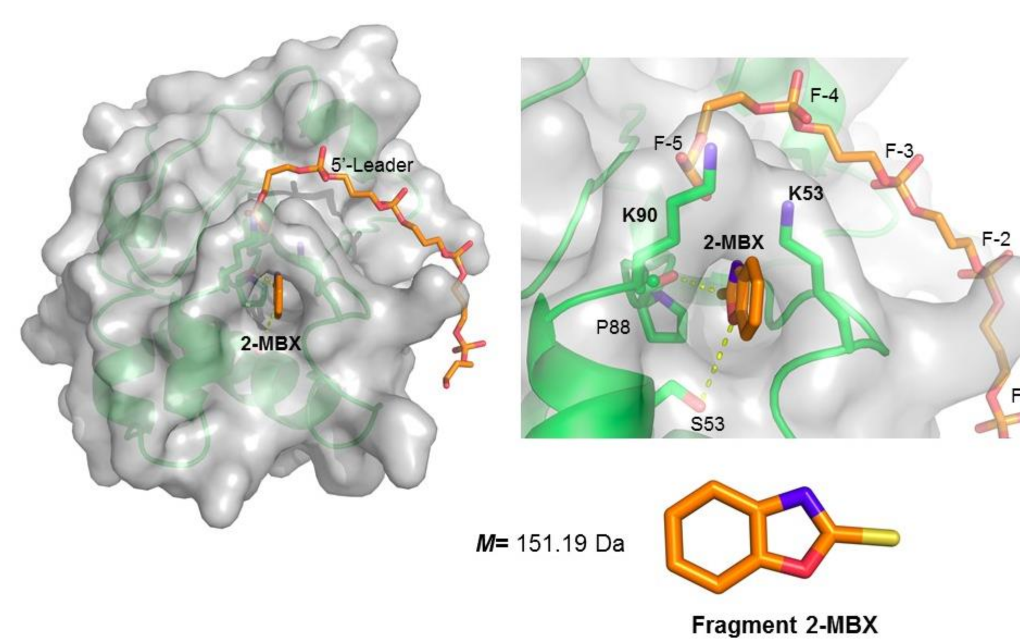


Figure 3. Crystal structure of P protein-2-MBX complex.

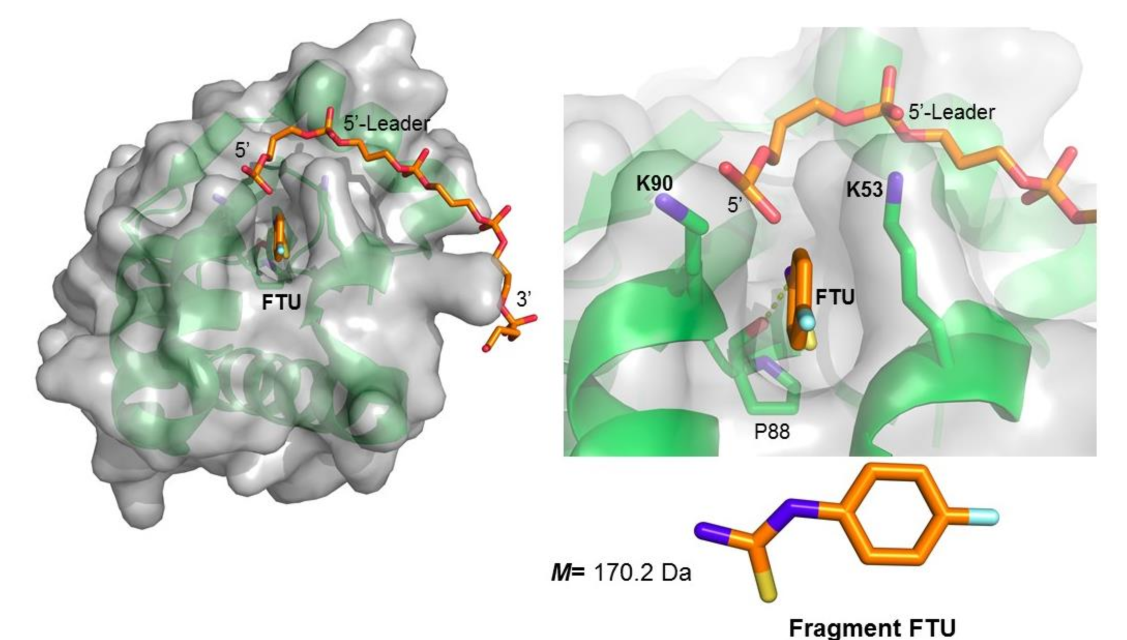


Figure 4. Crystal structure of P protein-FTU complex. FTU binding site is the exact same as that of fragment 2-MBX; evidence of the existence of a hot spot for small molecules in this shallow cavity of the P protein subunit.

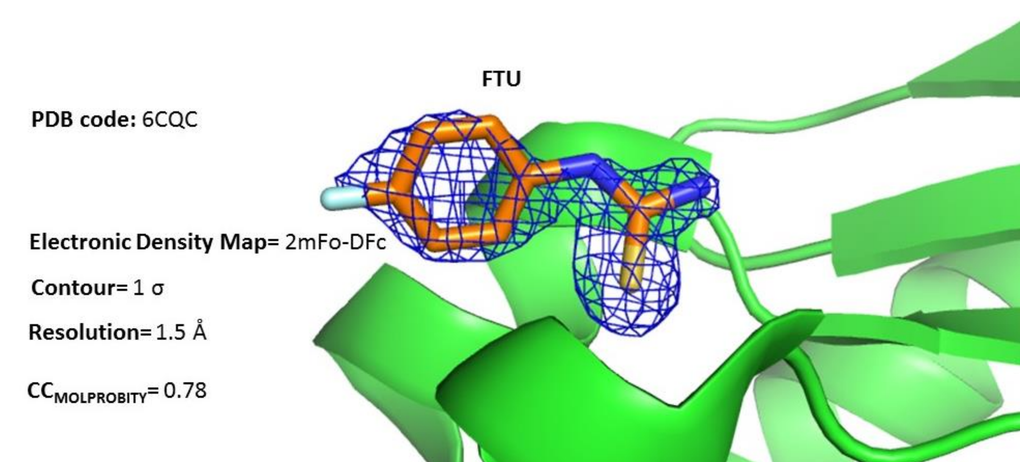


Figure 5. Electron density of fragment FTU. Ligand FTU was detected with the PanNNDa software. PDB accesión code: 6CQC.

During a Fragment-Based Drug Discovery campaign, weak-affinity, low-occupancy ligands are expected and are missed by conventional crystallographic methods. Because of this reason, we applied the Pan-Dataset Density Analysis method (PanDDA) (4), which carries out a statistical analysis of multiple crystallographic data sets that allows to contrast the electronic density of different background states and localize regions in the difference map that present a binding event.

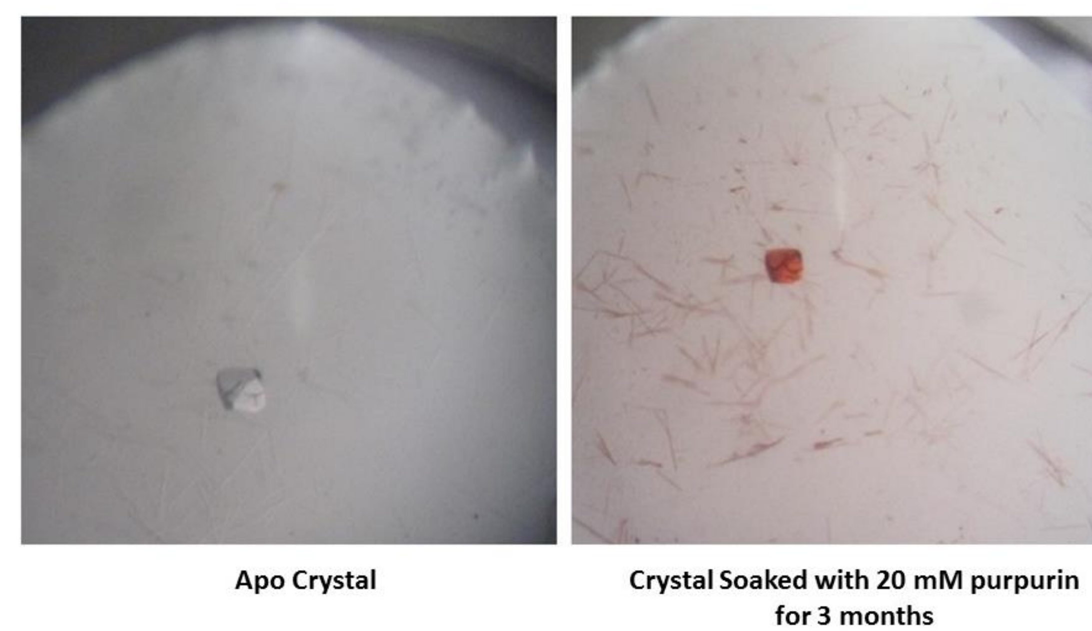


Figure 6. **Crystal of P protein soaked with purpurin inhibitor.** An all-polyethylenglycol regime for crystallognesis and soaking experiments was planned allowing minimal crystal perturbation. Mother liquor: PEG-1000, 12%; dilution of compounds: PEG-400, 50%; and cryoprotector solution: PEG-1000, 35%. Purpurin was soaked at 20 mM for 3 months.

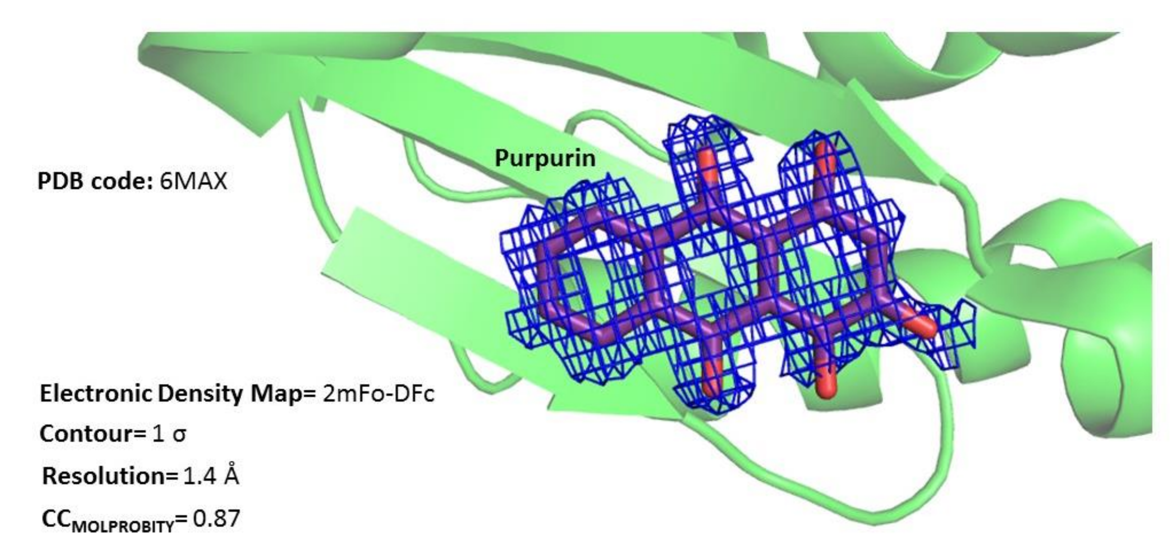


Figure 7. Electron density of purpurin inhibitor. Electron density map 2mFo-DFc at 1 σ contour of compound 1,2,4-Trihydroxyanthraquinone (purpurin) was detected in The Spectrum Collection small molecule library. PDB accesión code: 6MAX.

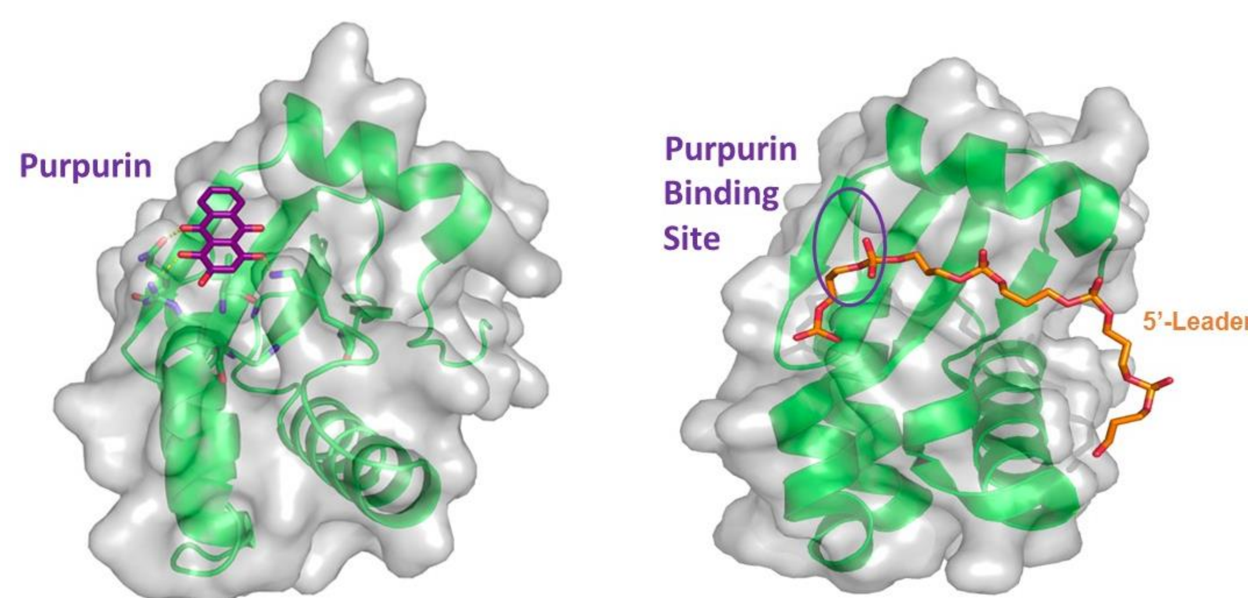


Figure 8. Purpurin binds in the 5' leader binding site. The inhibitor sterically blocks the interaction of the P protein subunit with the 5 pre-tRNA 5' leader sequence.

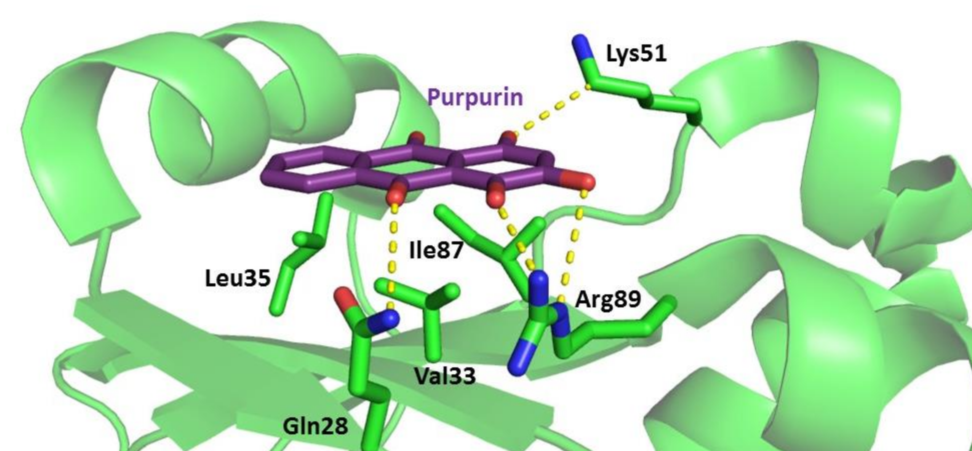
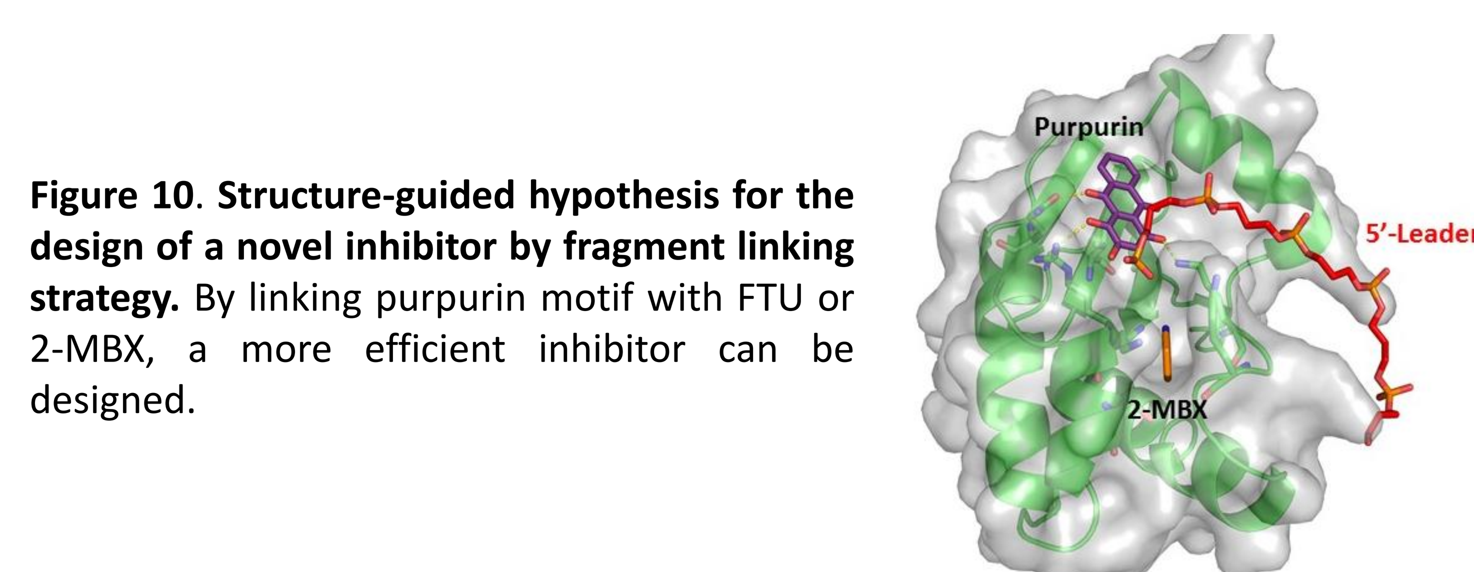


Figure 9. Purpurin inhibitor binding mode. The P protein subunit recognizes the purpurin inhibitor through three hydrophylic interactions: Gln28, Lys51 and Arg89; and three hydrophobic interactions: Val33, Leu35 and Ile87.

Figure 10. **Structure-guided hypothesis for the design of a novel inhibitor by fragment linking strategy.** By linking purpurin motif with FTU or 2-MBX, a more efficient inhibitor can be designed.



Conclusions

The strategies developed in this work, as well as the lead compounds detected will further the knowledge of the design of novel wide-spectrum antibacterial agents against RNase P.

References

1. Guerrier-Takada, *et al*. Cell (1983). **35**, 849-857.
2. Reiter, *et al*. Nature (2010). **468,** 784-791.
3. Madrigal-Carrillo, *et al*. Nucl Ac Res (2019). **47(12),** 6425-6438.
4. Pearce, *et al.* Nat Commun (2017). **8:**15123.