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ANTIBACTERIAL COMPOUNDS TARGETING THE DNA POLYMERASE III HOLOENZYME

The improper use of modern antibiotics in the treatment of human disease, and their widespread application in agriculture, has led to the development of antibiotic-resistant bacteria. Drug-resistant infections are already responsible for more than half million deaths globally each year. To generate new antibiotics with a broad spectrum of action, of particular interest are the protein-protein interactions (PPIs) as new molecular target considering their key role in most cellular processes, under both physiological and pathological conditions.

The aim of this project is the identification for small molecules capable to inhibit the PPI between β-slinding clamp and α subunit of the bacterial DNA polymerase III holoenzyme with an *in vivo* high-throughput platform based on the Bioluminescence Resonance Energy Transfer (BRET) technology. The screening of the small molecule libraries aims to identify "hit compounds" capable of interacting and modulating the biological activity of the target of interest and therefore of interfering with replication complex assembly, thus blocking pathogen viability and propagation. Furthermore, bioinformatic analysis have highlighted a consensus sequence (QL[S/D]LF) that is conserved also in other proteins interacting with the β-sliding clamp.

To this end, I reproduced the interactions between β-sliding clamp—α and β-sliding clamp—consensus in the unicellular eukaryote *Saccharomyces cerevisiae* and monitored them using the yeast-BRET (yBRET) assay. For this experiment NanoLuc luciferase (Nluc) was used as a donor, linked to the N- or to the C-terminal end of the α subunit and consensus, while Yellow Fluorence Protein (YFP) was used as an acceptor and bound to the N- or to the C-terminal end to the β-sliding clamp. The analysis of the relative orientation of BRET partners showed that the highest BRET signal was detected when the Nluc was fused at the N-terminal end of both α subunit or consensus, and when the YFP was fused at C-terminal of β-sliding clamp. BRET signal specificity was thus investigated with two different experiments: i) the donor saturation assay, which allows to distinguish between a specific signal and a signal deriving from random interactions, by monitoring the BRET signal at increasing acceptor fusion protein concentrations, and ii) a mutagenesis assay, in which the key residues for the interactions were mutated in α subunit and consensus sequences. These experiments indicated that the BRET signal was specific for the two reproduced interactions.

Subsequently I screened two untargeted small molecule libraries, for a total of over 6,600 different compounds, with the interaction β-sliding clamp-YFP—Nluc-consensus (which gave the highest BRET signal). Hit compounds emerging from yBRET need to be validated with ELISA assay to confirm their ability to disrupt

the α—β-sliding clamp interaction. Therefore, I expressed the fusion proteins β-sliding clamp-MycTag, GST-consensus, and α subunit in the prokaryotic organism *Escherichia coli.* The GST is needed to anchor the consensus peptide at the ELISA's well, while the MycTag is needed for the detection of the PPI with secondary antibodies. The first two proteins were successfully purified by FPLC, while α subunit expression needs to be optimized to solubilize the recombinant protein.