Riboswitches are RNA elements that regulate gene expression by changing their structure upon binding small molecule ligands in their environment. Development of synthetic riboswitches for use as molecular biosensors remains an active area of research [1]. Contemporary understanding of riboswitch function highlights the complex mechanisms by which they regulate gene expression. Some mechanisms, such as those elucidated for the *Escherichia coli lysC* riboswitch, are dual-acting to prevent translation initiation and trigger mRNA decay [2]. Others, like those of the *N. crassa* NMT1 riboswitch, use alternative splicing to regulate the development of mature mRNA [3]. This project focuses on the *E. coli thiB* TPP riboswitch, which represses gene expression when exposed to thiamine pyrophosphate (TPP). We investigate the hypothesis that this riboswitch functions through an RNA strand invasion mechanism. This was tested through site-directed mutagenesis of functionally critical parts of the riboswitch, such as the ligand binding aptamer region and the invading strand sequence. In-vivo gene expression assays were utilized to determine the minimal structures necessary for the riboswitch to conduct efficient regulation. It was found that in absence of TPP, the strand invasion mechanism occurs. Here, the invading sequence base pairs with nucleotides in the aptamer region, which prevents sequestering of the ribosome binding site (RBS) to allow gene expression. In high concentrations of TPP, the *thiB* riboswitch adopts a stable conformation which blocks strand invasion, thereby inhibiting translation initiation. With further understanding of their common regulatory pathways, the engineering of synthetic riboswitches as molecular biosensors will be more reasonable.

References