Split-Broc: An *In Vivo* Demonstration of a Split Fluorescent Aptamer. K. K. Alam,[1,2] K. D. Tawiah,[1,2] M. F. Lichte,[1,2] D. Porciani[2,3] & D. H. Burke[1,2,3,4], [1]Department of Biochemistry, University of Missouri, Columbia, MO 65211, United States, [2]Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, United States, [3]Department of Molecular Microbiology & Immunology, University of Missouri, Columbia, MO 65212, United States, [4]Department of Bioengineering, University of Missouri, Columbia, MO 65211, United States

Introduction: RNA-RNA interaction dynamics underlie important cellular functions and research tools in modern biology, and they likely served important roles in any early RNA-based life. For example, large functional RNAs can be assembled from multiple small RNAs, which can be more readily synthesized by polymerase ribozymes. In addition, the ability for RNAs to interact allows for a faster time scale and lower energy cost than protein interaction. However, it is difficult to monitor these interactions directly. Instead, it is common to infer RNA hybridization indirectly by linking the event to the translation of a fluorescent protein. We have developed a fluorescence-based assay based on a split version of a dye-binding aptamer that allows direct visualization of RNA-RNA hybridization events without the need for fluorescent protein. This construct also demonstrates an RNA-only logic gate that has its own output signal and can turn on/off gene expression in vivo.

The split aptamer design combines two copies of "Broccoli" (an RNA aptamer that activates fluorescence from the dye DFHBI-1T) and a three-way junction (3WJ) motif to generate 3WJ-double-Broccoli (3WJdB). This aptamer was then split into two strands that, when separated, do not effectively bind to the dye but, when put in solution together, they anneal and allow for dye binding and visual output (Split-Broc). Split-Broc was tested *in vitro* and *in vivo* and found to be successful at giving rapid signal for an RNA-only AND gate. Split-Broc was then applied to a synthetic circuit system that utilized a trigger/toehold mechanism to control gene expression. By combining the systems of Split-Broc and trigger/toehold, we created a circuit that had both green and red fluorescent output that was verified to be a result of RNA hybridization. This system therefore demonstrates assembly of complex molecular devices through RNA-RNA hybridization both in vitro and in cells, analogous to quaternary assembly of protein complexes.