Conditionally Stabilized Chimeric Aptamer Biosensors. Matthew F. Lichte,[1,2] Jordyn Lucas[1,2] & Donald 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: Communication between RNA molecules and the metabolome would have been a critical component of an RNA world, governing substrate utilization by ribozymes, allosteric regulation, and other processes. We would like to explore the evolution of RNA-metabolome interactions within living cells, but it is currently not straightforward to monitor such binding events. To address this problem, we are developing direct sensors for RNA binding to specific metabolites that allow for more real time monitoring of their concentration or presence without the lag from generating fluorescent protein or inducing other pathways. Specifically, we constructed a series of chimeras that fuse a metabolite-binding aptamer that serves as a Sensor module and a dimeric version of the Broccoli aptamer ("dBroc"), which activates fluorescence from the dye DFHBI-1T and serves as a reporter module. The system is designed to be unstable and non-fluorescent in the absence of the target metabolite. Binding of the metabolite to the Sensor module stabilizes the Broccoli aptamer by changing the RNA secondary structure, thereby allowing it to bind to the dye and fluoresce. The concept of the sensor relies on the change in DeltaG of the ligand-binding aptamer that accompanies the binding its respective ligand. This event will stabilize the RNA only under certain conditions to allow binding to the dye. Not only does this create an effective biosensor, but it is also a demonstration of RNA's ability to be conditionally active driven by a conformational change rather than a switch in annealing domains. We are currently testing the function and evolution of these sensors in vitro and in living cells, in addition to evaluating their use in optimizing ribozymes for RNA-catalyzed metabolic pathways. We will present the results at AbSciCon 2017.