Washington University in St. Louis

Washington University Open Scholarship

Volume 12

Washington University Undergraduate Research Digest

Spring 2017

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# Designing Ultrasensitive Responses in Cellular Systems by Introducing Cooperativity into Artificial Riboswitches

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An interest in synthetic biology is creating systems with higher sensitivity to stimulus, which allows for response thresholds to be achieved in biosensors and systems to behave in a more predictable manner. One way in which nature elicits this response is through cooperativity, the biochemical principle in which the binding of a ligand increases the rate of subsequent ligand binding events. This research focused on introducing cooperativity into the toehold riboswitch, an artificial and versatile post-transcriptional form of gene regulation. Toehold switches are a form of RNA secondary structure that forms upstream of a gene, sequestering the ribosome binding site in the absence of a trans-acting RNA. By introducing cooperativity, we hope to not only achieve higher degrees of stimulus sensitivity, but also to test the limits of how well RNA behavior can be designed through theoretical computer simulations. By using the RNA simulation program NUPACK, additional ligand binding sites were rationally introduced into the original riboswitch design. A model was then generated with parameters taken from literature to show that theoretical results from these modifications would yield higher sensitivity. The riboswitches were then cloned upstream of a fluorescent reporter protein and downstream of a constitutive promoter using inverse PCR. The trans-activating RNAs were similarly cloned downstream of inducible promoters. These two vectors were transformed into *E. coli* and inducer concentration transfer curves were experimentally constructed. These transfer curves were then fitted to the general Hill equation to obtain general Hill coefficients which were used to quantitatively measure cooperativity in the system. It was found that an increase in the number of ligand binding sites decreased the absolute fluorescence but increased the general Hill coefficient, which may be indicative of the presence of cooperativity.