

## **Development of regulated RNA aptamers as tools for *in vivo* post-genomic analysis.**

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There is a great deal of interest in naturally occurring RNA domains that can lead to direct genetic regulation by the small molecular weight products of biosynthetic pathways – “riboswitches”. Our long term goal is to create a generic version of these regulatory systems that can be “switched” between off- and on-states by the addition to the cellular growth medium of small molecular weight effector molecules. This strategy will allow critical issues of the roles of specific macromolecular interactions throughout cellular growth and development to be addressed in our test model organism *C. elegans*.

As a proof of principle of this idea, we have used the *E. coli* methionine apo-repressor, MetJ, as a model system. We used a number of different approaches for selection of aptamers against MetJ, including robotic selection on a Biomek 2000 Automated Workstation, gel-retardation and filter-binding assays. Isolated RNA aptamers bind to MetJ with  $K_d$ 's  $\sim$ 5 nM, and were able to interfere efficiently with MetJ binding to promoter DNA *in vitro*. In order to create an allosterically-regulated aptamer against MetJ, we tested a library of novel aminoglycoside derivatives for antimicrobial activity against an *E. coli* reference strain. Five such aminoglycoside compounds from the library that did not show antibiotic activity were chosen as the potential effector molecules, and we have begun the selection of aptamers against these compounds. Once tight binding aminoglycoside domains are to hand, the next step will be to combine the two aptamer sequences, anti-target aptamer, and effector-binding aptamer, into the same RNA molecule, followed by further selection to obtain allosteric coupling. Such RNA aptamers would have great potential in regulation of protein interactions in response to the addition of small molecular weight effector.

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