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Sensitive and Specific Detection of Ligands Using Engineered Riboswitches

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Highlights

- A guanine riboswitch is engineered for use as an *in vitro* sensor for guanine
- Hybrid sensors for 2'-deoxyguanosine and cyclic-diGMP are produced
- A simple method for the *in vitro* selection of sensors with novel ligand specificity is described

ABSTRACT

Riboswitches are RNA elements found in non-coding regions of messenger RNAs that regulate gene expression through a ligand-triggered conformational change. Riboswitches typically bind tightly and specifically to their ligands, so they have the potential to serve as highly effective sensors *in vitro*. In *B. subtilis* and other gram-positive bacteria, purine nucleotide synthesis is regulated by riboswitches that bind to guanine. We modified the *xpt-pbuX* guanine riboswitch for use in a fluorescence quenching assay that allowed us to specifically detect and quantify guanine *in vitro*. Using this assay, we reproducibly detected as little as 5 nM guanine. We then produced sensors for 2'-deoxyguanosine and cyclic diguanylate (c-diGMP) by appending the P1 stem of the guanine riboswitch to the ligand-binding domains of a 2'-deoxyguanosine riboswitch and a c-diGMP riboswitch. These hybrid sensors could detect 15 nM 2'-deoxyguanosine and 3 nM c-diGMP, respectively. Each sensor retained the