

Electronic Supplementary Information for Chemical Communications

Synthetic mammalian riboswitches based on guanine aptazyme

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Materials and Methods

Plasmid construction

All plasmids were prepared by standard recombinant DNA techniques. Plasmids encoding the *trans*-acting (RNAi) riboswitches and the controls (RzGuaM1-miREGFP, pRzGuaM5-miREGFP, pL22, pE22) were derived from pSilencer 2.1-U6 hygro from Ambion. The riboswitch expression is driven by the U6 promoter. Plasmids encoding the *cis*-acting (3' UTR) riboswitches were derived from pEGFP-N1 (Clontech). Appropriate aptazyme sequences were cloned in the 3' UTR of the EGFP transcript. Nucleotide sequences of the key regions are provided elsewhere in this ESI.

Cell culture and transfection

HEK293 cells were maintained in a 5% CO₂ humidified incubator at 37°C in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum (FBS) (JR Scientific) and 1× antibiotic-antimycotic (Invitrogen). One day before transfection, HEK293 cells were trypsinized and diluted appropriately with fresh complete medium, and 2.4 × 10⁴ cells/well (~100 µl) were seeded onto 96-well plates. Fifty-five nanograms of *cis*-acting riboswitch (3'UTR-RzGuaM1/5) or control (pEGFP-N1) plasmid, 55 ng of *trans*-acting riboswitch (RzGuaM1/5-miREGFP) or control (pL22 / pE22) plasmid, 10 ng of pCMV-mCherry plasmid, were cotransfected using 1 µl of PolyFect reagent (QIAGEN) per well according to the manufacturer's instruction. After 3.5 hr incubation, the media were removed and replaced with 100 µl fresh complete media containing appropriate concentrations of guanine. Guanine (Acros) was first dissolved in 100× concentrations in 0.2 M NaOH, and was diluted 100-fold with the complete medium immediately before use. The cell were incubated for additional 20 hr before fluorescence measurement.

Fluorescence measurements

Cellular fluorescence was measured and normalized according to our previous report (D. Kumar, C.-I. An and Y. Yokobayashi, *J. Am. Chem. Soc.*, 2009, **131**, 13906-13907.). Briefly, the cell culture media were replaced with phosphate buffered saline (PBS) (150 µl per well) and incubated at 37 °C for 10 min. Fluorescence intensities were measured for EGFP (484 nm excitation / 510 emission / 5 nm bandwidth) and mCherry (587 nm excitation / 610 nm emission / 10 nm bandwidth) using Safire2 microplate reader (Tecan). The raw fluorescence values were first subtracted with that of the untransfected cells (background). For each well, EGFP fluorescence was normalized by mCherry ([EGFP fluorescence] / [mCherry fluorescence]) to account for variations in transfection efficiency. The values were further normalized by the pL22/pEGFP-N1/pCMV-mCherry transfected cells (=1.0). The reported values are mean ±SD from at least three replicate samples.

MTT assay

MTT assay was performed using Cell Proliferation Kit I (Roche). A 96-well plate was seeded with 2.4 × 10⁴ cells/well and cultured for 24 hr. The media were replaced with fresh media with or without guanine (500 µM) and cultured for additional 18 hr and assayed according to the manufacturer's instruction. The reported values are mean ±SD from five replicate samples.

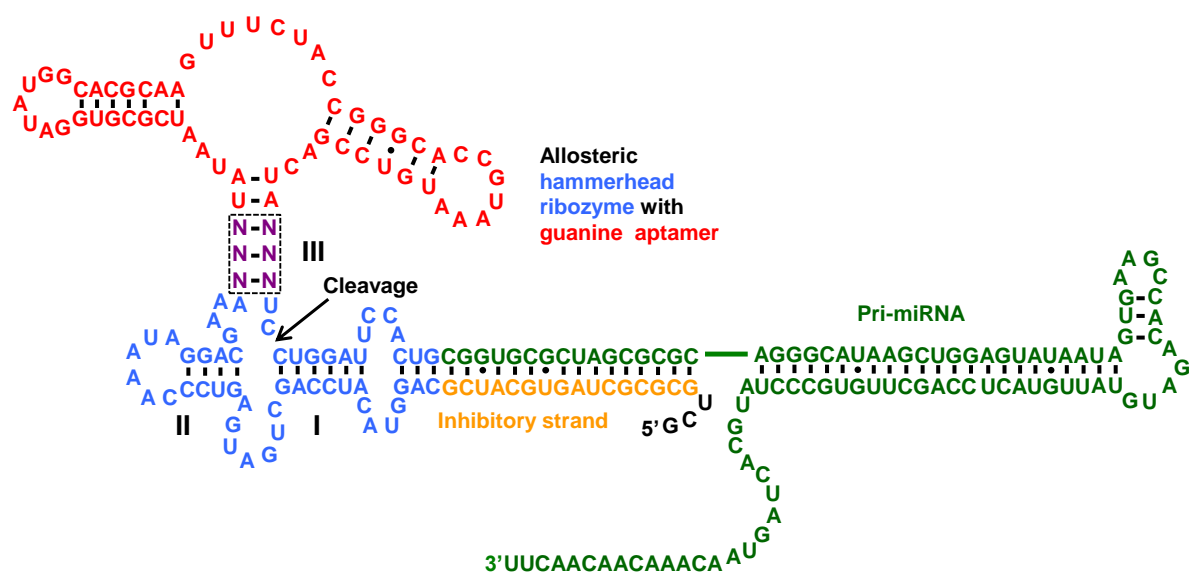


Fig. S1 Full sequence and architecture of the *trans*-acting riboswitches (RzGuaM1/5-miREGFP).

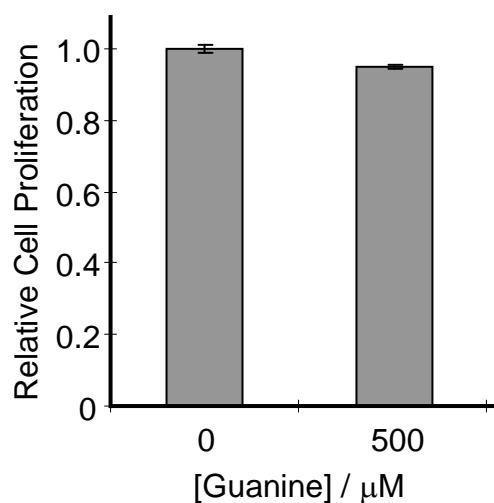


Fig. S2 Cell proliferation assay (MTT assay). HEK293 cells were cultured in the absence and presence (500 μM) of guanine and subjected to MTT assay as described in Materials and Methods. The reported values are mean \pm SD from five replicate samples.

Riboswitch sequences

Partial sequences of the predicted transcripts that include the riboswitch sequences are listed below. Full plasmid sequences are available from the authors upon request.

Cis-acting riboswitches and controls (3' UTR)

3' UTR of EGFP-N1

5' -**ACAAGUAA**AGCGGCCGCGACUCUAGAUCUAAAUCAGCCAUACCACAUUUGUAGAGGUUUU
ACUUGC UUUAAAAACCUC CACACCUC CCCCUGAAC CUGAAACA UAAAAUGAAUGCAAUUGU
UGUUGUUAACUUGUUUAUUGCAGCUUAUAAUGGUUACA**AAUAAA**GCAAUAGCAUCACAAAUUU
CAC**AAUAAA**G-3'

3' UTR-RzGuaM1

5' -**ACAAGUAA**AGCGGCCGCGACUCUAGAGCG**CAGGUACA**UCCAGCUGAUGAGUCC**AAA**UAG
GACGAAA**UAC**UAUAAUCGCGUGGAUAUGGCACGCAAGUUUCUACCGGGCACCGU**AAA**UGUCCG
ACUAGUAUCCUGGAU**UCCACUG**CGCUC CCGAGCCAUACCACAUUUGUAGAGGUUUUACUUGC
UUAAAAACCUC CACACCUC CCCCUGAAC CUGAAACA UAAAAUGAAUGCAAUUGUUGUUGU
AACUUGUUUAUUGCAGCUUAUAAUGGUUACA**AAUAAA**GCAAUAGCAUCACAAAUUUCACA**AAU**
AAAG-3'

3' UTR-RzGuaM5

5' -**ACAAGUAA**AGCGGCCGCGACUCUAGAGCG**CAGGUACA**UCCAGCUGAUGAGUCC**AAA**UAG
GACGAAA**CAC**UAUAAUCGCGUGGAUAUGGCACGCAAGUUUCUACCGGGCACCGU**AAA**UGUCCG
ACUAUUGUCCUGGAU**UCCACUG**CGCUC CCGAGCCAUACCACAUUUGUAGAGGUUUUACUUGC
UUAAAAACCUC CACACCUC CCCCUGAAC CUGAAACA UAAAAUGAAUGCAAUUGUUGUUGU
AACUUGUUUAUUGCAGCUUAUAAUGGUUACA**AAUAAA**GCAAUAGCAUCACAAAUUUCACA**AAU**
AAAG-3'

3' UTR-Rz*GuaM5 (A-to-G ribozyme inactivation mutation underlined)

5' -**ACAAGUAA**AGCGGCCGCGACUCUAGAGCG**CAGGUACA**UCCAGCUGAUGAGUCC**AAA**UAG
GACGAG**A****CAC**UAUAAUCGCGUGGAUAUGGCACGCAAGUUUCUACCGGGCACCGU**AAA**UGUCCG
ACUAUUGUCCUGGAU**UCCACUG**CGCUC CCGAGCCAUACCACAUUUGUAGAGGUUUUACUUGC
UUAAAAACCUC CACACCUC CCCCUGAAC CUGAAACA UAAAAUGAAUGCAAUUGUUGUUGU
AACUUGUUUAUUGCAGCUUAUAAUGGUUACA**AAUAAA**GCAAUAGCAUCACAAAUUUCACA**AAU**
AAAG-3'

- █ EGFP coding region (3' end)
- █ Hammerhead ribozyme
- █ Stem III connecting the ribozyme and the aptamer
- █ Guanine aptamer
- █ SV40 early mRNA polyadenylation signal

Trans-acting riboswitches and controls (RNAi)

RzGuaM1-miREGFP

5' -GCUGCGCGCUAGUGCAUCGCAGGUACAUCCAGCUGAUGAGUCCCAAUAGGACGAAUAC
UAUAAUCGCGUGGAUAUGGCACGCAAGUUUCUACCGGGCACCGUAAAUGUCCGACUAGUAUCC
UGGAUUCACUGCGGUGCGCUAGCGCGCA GGGCAUAAGCUGGAGUAUAAUAGUGAAGCCACAG
AUGUAUUGUACUCCAGCUUGUGCCCUAUGCACUAGUAACAAACAACAACUU-3'

RzGuaM5-miREGFP







5' -GCUGCGCGCUAGUGCAUCGCAGGUACAUCCAGCUGAUGAGUCCCAAUAGGACGAAACAC
UAUAAUCGCGUGGAUAUGGCACGCAAGUUUCUACCGGGCACCGUAAAUGUCCGACUAUUGUCC
UGGAUUCACUGCGGUGCGCUAGCGCGCA GGGCAUAAGCUGGAGUAUAAUAGUGAAGCCACAG
AUGUAUUGUACUCCAGCUUGUGCCCUAUGCACUAGUAACAAACAACAACUU-3'

pE22

5' -GGAACAACAGACAGCUAGCGCGCA GGGCAUAAGCUGGAGUAUAAUAGUGAAGCCACAGAU
GUAUUGUACUCCAGCUUGUGCCCUAUGCACUAGUGGAACAACAGACAUU-3'

pL22

5' -GGAACAACAGACAGCUAGCGCGCA GCACUUUGAUUGACAAUAAUAGUGAAGCCACAGAU
GUAGUAUUGUCAAU CAGAGUGCUAUGCACUAGUGGAACAACAGACAUU-3'

-  Inhibitory strand
-  Hammerhead ribozyme
-  Stem III connecting the ribozyme and the aptamer
-  Guanine aptamer
-  Sense (passenger) strand of pri-miRNA
-  Antisense strand of pri-miRNA