Supplementary Information for Chemical Communications

A synthetic riboswitch with chemical band-pass response

Norihito Muranaka, and Yohei Yokobayashi*

Experimental methods

Media and reagents

LB and M9 minimal media were prepared according to the standard procedures.[1] M9 medium was supplemented with 0.8 % glycerol (Fisher Scientific) as the carbon source and 0.1 % casamino acids (BD Biosciences). Agar (Fisher Scientific) was added at 1.5 % to prepare agar plates.

Plasmid preparation

All plasmids used to conduct experiments described in this study are derived from pLacT1[2] which contains a ColE1 origin of replication and an ampicillin resistance marker (*bla)*. First, the -thiC(wt/#19) riboswitch was amplified by polymerase chain reaction (PCR) using primers pbla-r (5'-ATTGT CTCAT GAGCG GATAC-3') and thiC-EcoRI-r (5'-CCGGA ATTCA AAAAG TTAAG GACGT GGC-3'), and p-thiC(wt/#19)-gfpuv[3] as a template. After digesting template plasmid with *Dpn* I (New England Biolabs), the PCR product was digested with *Xho* I and *Eco* RI. The digested fragment was cloned into *Xho* I-*Eco* RI site of pLac-thiMN$_{15}$#19-gfpuv[4] to obtain p-thiC(wt/#19)+thiMN$_{15}$#19-gfpuv. Second, the *thiC* promoter was replaced with the *tac* promoter by PCR using primers ptac-thiC-f (5'-CATCG GCTCG TATAA TGTGT GGAAT GCCCC ATTTG CGGGG CT-3') and ptac-r (5'-ATTAA TTGTC AACAG CTCAT TCTCG AGGTG AAGAC GAAAG GGC-3'), and p-thiC(wt/#19)+thiMN$_{15}$#19-gfpuv as a template. After digesting the template plasmid with *Dpn* I, the PCR product was column-purified by DNA Clean & Concentrator-5 (Zymo Research), and phosphorylated with T4 polynucleotide kinase (New England Biolabs). The phosphorylated linear DNA was self-ligated and transformed into *E. coli* TOP10 cells (Invitrogen). The transformed cells were plated on LB agar plates and incubated overnight at 37 °C. The pTac-thiC(wt/#19)+thiMN$_{15}$#19-gfpuv shown in Figure S2 was isolated from a colony using Zippy Plasmid Miniprep Kit (Zymo Research).

Fluorescence measurements

All cells grown in liquid media were incubated at 37 °C and 275 rpm in an incubator-shaker (New Brunswick Scientific I2400). *E. coli* TOP10 cells (Invitrogen) transformed with the appropriate plasmids in Figure 2 were plated on LB agar plates supplemented with ampicillin (Fisher Scientific, 100 μg/mL), and incubated overnight. Single colonies were inoculated in 1 mL liquid LB medium supplemented with ampicillin (LB-amp) dispensed in 16 mm glass tubes and grown overnight. The overnight cultures were diluted 100-fold in 1 mL of fresh M9 minimal medium supplemented with ampicillin (M9-amp) and an appropriate concentration of thiamine (Acros Organics), and cultured for 8 h. The cells (OD_{600}≈0.3) were harvested by centrifugation, washed with 200 μL phosphate-buffered saline (PBS), and resuspended in 200 μL PBS. Cell suspensions (200 μL) were transferred to a 96-well plate. GFPuv fluorescence (395 nm excitation, 509 nm emission) of the cells was measured by Safire2 microplate reader (Tecan). Wells filled with PBS were treated as background fluorescence and subtracted from the measured fluorescence intensities. The fluorescence intensity values were further normalized by the optical density measured at 600 nm (OD_{600}) to compensate for the variations in cell densities. The background fluorescence of cells transformed with a blank plasmid (pLac+thiMN_{15}#19-lacZ) was subtracted from all normalized fluorescence intensity values.

[1] J. Sambrook, D. W. Russel, *Molecular Cloning*. Cold Spring Harbor Laboratory: Cold Spring Harbor, N. Y., **2001**.

[2] Y. Nomura, Y. Yokobayashi, *Biosystems* **2007**, *90*, 115.

[3] N. Muranaka, Y. Yokobayashi, *Angew. Chem., Int. Ed.* **2010**, *in press*

[4] N. Muranaka, V. Sharma, Y. Nomura and Y. Yokobayashi, *Nucleic Acids Res*, **2009**, *37*, e39.

Figure S1. Thiamine-dependent GFPuv expression of –thiC#19 (closed circles), +thiMN_{15}#19 (open circles) and –thiCwt (closed triangles) characterized in the previous study.[3] All fluorescence data are averages of measurements from two independent cultures (8h after dilution) and the error bars represent the range of the two measurements. The fluorescence intensities were normalized to the observed fluorescence of –thiC#19 grown without thiamine (=1.0). Curves are shown to guide the eye only.

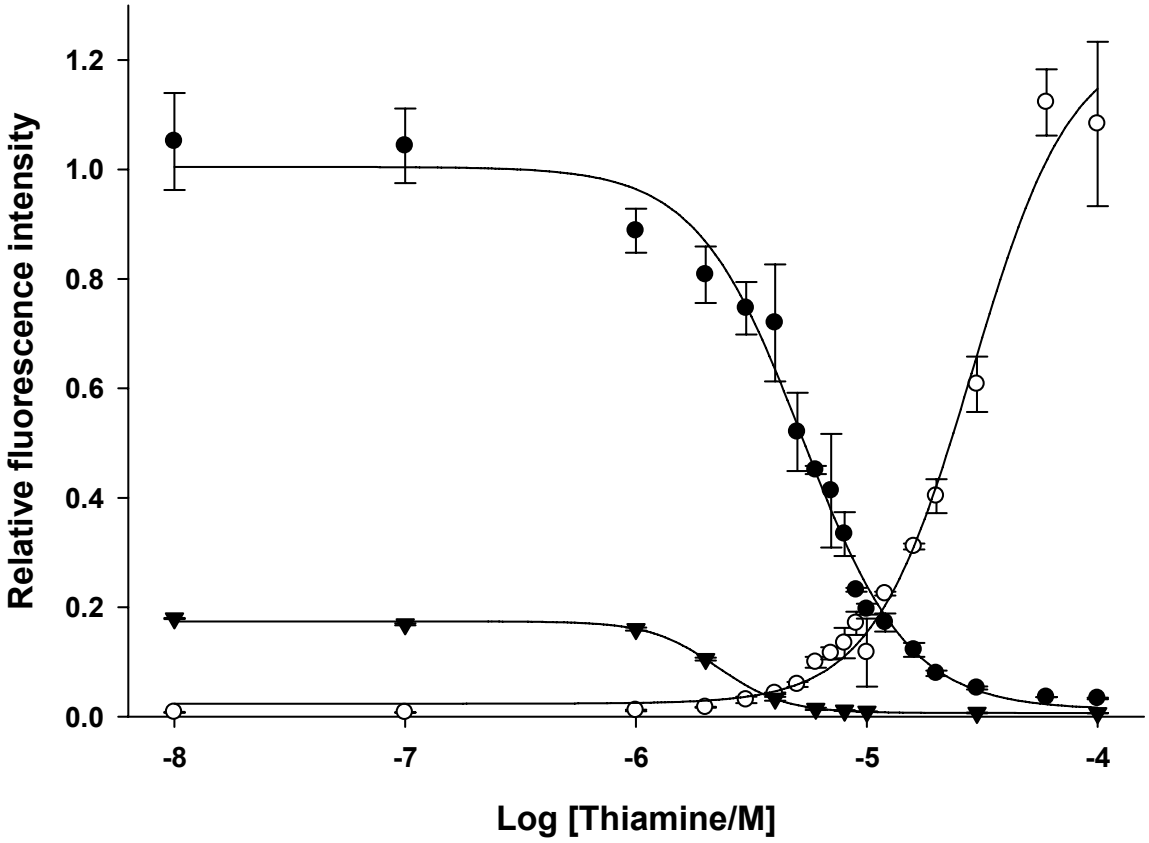
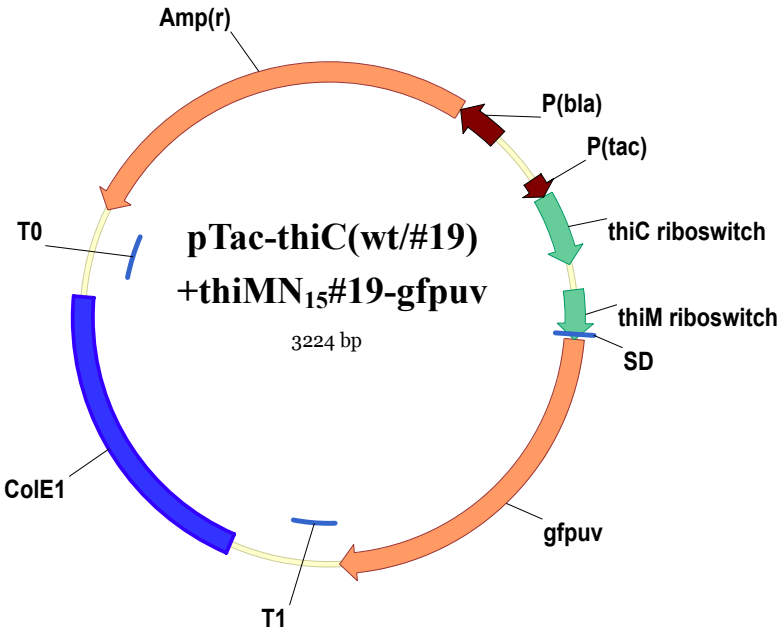


Figure S2. Design of the plasmids used in this study. a) Plasmid map. b) DNA sequences of the key regions within the plasmids.

a)



b) **pTac-thiCwt+thiMN$_{15}$#19-gfpuv (mutations from the wild-type riboswitch are shown in red)**

P(tac) -35 -10 +1

GAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATGCCCCATTTGCGGGG

ThiC TPP aptamer

CTAATTTCTTGTCGGAGTGCCTTAACTGGCTGAGACCGTTTATTCGGGATCCGCGGAA

CCTGATCAGGCTAATACCTGCGAAGGGAACAAGAGTTAATCTGCTATCGCATCGCCC

CTGCGGCGATCGTCTCTTGCTTCATCCGTCGTCTGACAAGCCACGTCCTTAACTTTTTG

ThiM TPP aptamer

AATTCAACCAAACGACTCGGGGTGCCCTTCTGCGTGAAGGCTGAGAAATACCCGTATC

Selected sequence SD sequence

ACCTGATCTGGATAATGCCAGCGTAGGGAAGCTCTTAAGAATCAGATC*AGGAG*CAAA

Start codon (*ThiM*) (*gfpuv*)

CTATGCAAGTCGACCTGCTGGATCCATTGAGTAAAGGA----------

pTac-thiC#19+thiMN$_{15}$#19-gfpuv (mutations from the wild-type riboswitch are shown in red)

P(tac) -35 -10 +1

GAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATGCCCCATTTGCGGGG

ThiC TPP aptamer

CTAATTTCTTGTCGGAGAGCCTTAATTGGCTGAGACCGTTTATTCGGGATCCGCGGAA

CCTGATCAGGCTAATACCTGCGAAGGGAACAAGAGTTAATCTGCTACCGCAACGCCC

CTGCGGCGATCGTCTCTTGCTTCATCCGTCGTCTGACAAGCCACGTCCTTAACTTTTTG

ThiM TPP aptamer

AATTCAACCAAACGACTCGGGGTGCCCTTCTGCGTGAAGGCTGAGAAATACCCGTATC

Selected sequence SD sequence

ACCTGATCTGGATAATGCCAGCGTAGGGAAGCTCTTAAGAATCAGATC*AGGAG*CAAA

Start codon (*ThiM*) (*gfpuv*)

CTATGCAAGTCGACCTGCTGGATCCATTGAGTAAAGGA----------