Electronic Supplementary Information

For

# Biofunction-assisted aptasensors based on ligand-dependent 3' processing of a suppressor tRNA in a wheat germ extract

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**CONTENTS:**

**Preparation of DNA templates.** DNA templates for *in vitro* transcription were prepared with standard polymerase chain reactions (PCRs) by using PrimeSTAR Max DNA Polymerase, which has extremely high fidelity, from Takara Bio (Ohtsu, Japan). Most of the DNA templates (other than those for **t86** and **3pt**) were constructed using sequential PCRs in which all the PCR products except for those in the final PCR were agarose gel-purified and then used as a template in the next PCR. The PCR template in the first PCRs for tRNA probes and primers were synthesized (and purified with an oligonucleotide purification cartridge for reverse primers in the final PCRs) by Life Technologies (Tokyo, Japan) or Eurofins Genomics (Tokyo, Japan). A reverse primer modified with 2'-methoxy-G (2'-OMe-G) at the second base from the 5' terminus was purchased from Tsukuba Oligo Service (Tsukuba, Japan) and used to prepare a template of the mature sup-tRNA (**t86**) to reduce the non-templated 3' nucleotide addition.[1] A PCR template for **amber-mRNA(FLuc)** was prepared by *Spe*I ligation between the 5' segment and the 3' segment, both of which were PCR-amplified from a plasmid named pE01-Luc encoding the firefly luciferase (FLuc) gene,[2] and the subsequent final PCR. The sequences of the PCR templates and primers are summarized below.

**Sequences of primers and templates for PCR.**

(written from 5' (left) to 3' (right); underlined: an *Spe*I site; X: 2'-OMe-G.)

Forward primer in the 5' segment amplification for **amber-mRNA(FLuc)**

ATAAGGGCGACACGGAAATGTTG

Reverse primer in the 5' segment amplification for **amber-mRNA(FLuc)**

GACACTACTAGTATGGAACATCACCATCACCATCATTAGAGATACGAAGACGCCAAAAAC
ATAAAGAAAG

Forward primer in the 3' segment amplification for **amber-mRNA(FLuc)**

GACACTACTAGTGATATCTTGGTG

Reverse primer in the 3' segment amplification and the final PCR for **amber-mRNA(FLuc)**

AGATAAACAGTATTTTGTCCAGTCGTCGAAC

Forward primer in the final PCR for **amber-mRNA(FLuc)**

CATACGATTTAGGTGACACTATAG

Template for all tRNA probes in the 1st PCRs

GGAGAGATGGCTGAGTGGTTGATAGCTGCGGTCTCTAAAACCGCTATAGTTCTAGGAACTA
TCGAGGGTTCGAATCCCT

Forward primer for all tRNA probes in the 1st PCRs

GAAATTAATACGACTCACTATAGGAGAGATGGCTGAGTG

Forward primer in the 2nd PCRs and 3rd PCR

GAAATTAATACGACTCACTATAG

Reverse primer for **t86** in the 1st (final) PCR

TXGCGGAGAGAGAGGGATTCGAACCCTCGATAGTTC

Reverse primer for **3pt** in the 1st (final) PCR

AAGCAAACGGAGAGAGAGGGATTCGAACCCTCGATAGTTC

Reverse primer for **3pt-IS(15)** in the 1st PCR

AGTCTCTCCGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGGATTCGAACCCTCGATAGTT
C

Reverse primer for **3pt-IS(15)** in the 2nd (final) PCR

AGTCTCTCCGTTTGCTTGTTG

Reverse primer for **3pt-IS(14)~(9)** in the 1st PCRs

AGGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGGATTCGAACCCTCGATAGT

Reverse primer for **3pt-IS(14)** in the 2nd (final) PCR

AGCTCTCCGTTTGCTTGTTGTAAG

Reverse primer for **3pt-IS(13)** in the 2nd (final) PCR

AGTCTCCGTTTGCTTGTTGTAAG

Reverse primer for **3pt-IS(12)** in the 2nd (final) PCR

AGCTCCGTTTGCTTGTTGTAAG

Reverse primer for **3pt-IS(11)** in the 2nd (final) PCR

AGTCCGTTTGCTTGTTGTAAG

Reverse primer for **3pt-IS(10)** in the 2nd (final) PCR

AGCCGTTTGCTTGTTGTAAG

Reverse primer for **3pt-IS(9)** in the 2nd (final) PCR

AGCGTTTGCTTGTTGTAAG

Reverse primer for **theo(th1)-MS(3)~(6)** in the 1st PCRs

CAAGGGCCTTTCGGCTGGTATGCTCTCCGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGG
ATTCGAACCCTCGATAGT

Reverse primer for **theo(th1)-MS(3)** in the 2nd (final) PCR

GAGCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th1)-MS(4)** in the 2nd (final) PCR

AGAGCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th1)-MS(5)** in the 2nd (final) PCR

GAGAGCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th1)-MS(6)** in the 2nd (final) PCR

GGAGAGCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo-MS(3)~(6)** in the 1st PCRs

CAAGGGCCTTTCGGCTGGTATCTCTCCGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGGA
TTCGAACCCTCGATAGT

Reverse primer for **theo-MS(3)** in the 2nd (final) PCR

GAGCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo-MS(4)** in the 2nd (final) PCR

AGAGCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo-MS(5)** in the 2nd (final) PCR

GAGAGCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo-MS(6)** in the 2nd (final) PCR

GGAGAGCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th2)-MS(2)~(6)** in the 1st PCRs

AAGGGCCTTTCGGCTGGTATGGCTCTCCGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGG
ATTCGAACCCTCGATAGT

Reverse primer for **theo(th2)-MS(2)** in the 2nd (final) PCR

AGCCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th2)-MS(3)** in the 2nd (final) PCR

GAGCCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th2)-MS(4)** in the 2nd (final) PCR

AGAGCCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th2)-MS(5)** in the 2nd (final) PCR

GAGAGCCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **theo(th2)-MS(6)** in the 2nd (final) PCR

GGAGAGCCCTGCCAAGGGCCTTTCGGCTGGT

Reverse primer for **m-theo(th1)-MS(4)** in the 1st PCR

CTATGGCCTTTCGGCTGGTATGCTCTCCGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGG
ATTCGAACCCTCGATAGT

Reverse primer for **m-theo(th1)-MS(4)** in the 2nd (final) PCR

AGAGCCTGCCTATGGCCTTTCGGCTGGT

Reverse primer for **tc(th1)-MS(4)** in the 1st PCR

GATCGAAATCTGGTATGTTTTGCTCTCCGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGG
ATTCGAACCCTCGATAGT

Reverse primer for **tc(th1)-MS(4)** in the 2nd PCR

AGAGCAGGTGGTCGTATTCTTCACCTCTCCAGATCGAAATCTGGTATGTTTTG

Reverse primer for **tc(th1)-MS(4)** in the 3rd (final) PCR

AGAGCAGGTGGTCGTATTC

Reverse primer for **FMN(th1)-MS(4)** in the 1st PCR

CCTTCTGCCGAAGCATATCCTGCTCTCCGTTTGCTTGTTGTAAGCAAACGGAGAGAGAGGG
ATTCGAACCCTCGATAGT

Reverse primer for **FMN(th1)-MS(4)** in the 2nd (final) PCR

AGAGCCCTTCTGCCGAAGCATATCCT

**Preparation of mRNA.** Run-off transcription of the obtained DNA template for **amber-mRNA(FLuc)** was performed with an AmpliScribe SP6 High Yield Transcription Kit (CellScript, Madison, WI) according to the manufacturer's protocol. The transcribed mRNA was purified with an RNeasy MinElute Cleanup Kit (QIAGEN, Tokyo, Japan) and quantified by the absorbance at 260 nm.

**Preparation of tRNA probes.** tRNA probes were constructed by *in vitro* run-off transcription of their DNA templates with an AmpliScribe T7 High Yield Transcription Kit (CellScript) according to the manufacturer's protocol. The transcribed tRNAs were purified with a QIAquick Nucleotide Removal Kit (QIAGEN) and quantified by the absorbance at 260 nm.

**Ligand-dependent 3' processing of tRNA probes and amber suppression in WGE.** WEPRO1240 in a WEPRO1240 Expression Kit (CellFree Sciences, Matsuyama, Japan) was used as wheat germ extract (WGE). A mixture (10 μL) of tRNA probe (50 pmol), **amber-mRNA(FLuc)** (3 pmol for

translation or no mRNA for gel analyses), WEPRO1240 (2 μL), creatine kinase (final concentration: 40 ng/μL), and SUB-AMIX (final concentration: 1×), the latter three of which were included in the kit, was incubated at 26°C for 1 h in the presence of various concentrations of ligands.

**Luciferase assays.** The chemiluminescence intensities of translated FLuc with luciferin were measured as previously described.[3] Suppression efficiencies were evaluated with relative intensities compared to that of **3pt**. The standard deviations were calculated from four sets of experiments.

**Gel analyses.** An aliquot (2.5 μL) of the solution incubated in WGE as described above was resolved by 15% SDS-PAGE and stained with ethidium bromide.[4] The amounts of probes on the gel were estimated from their band intensities using Image J software (NIH) by referring to a calibration curve drawn with a serially-diluted non-incubated probe.

**Figures.**

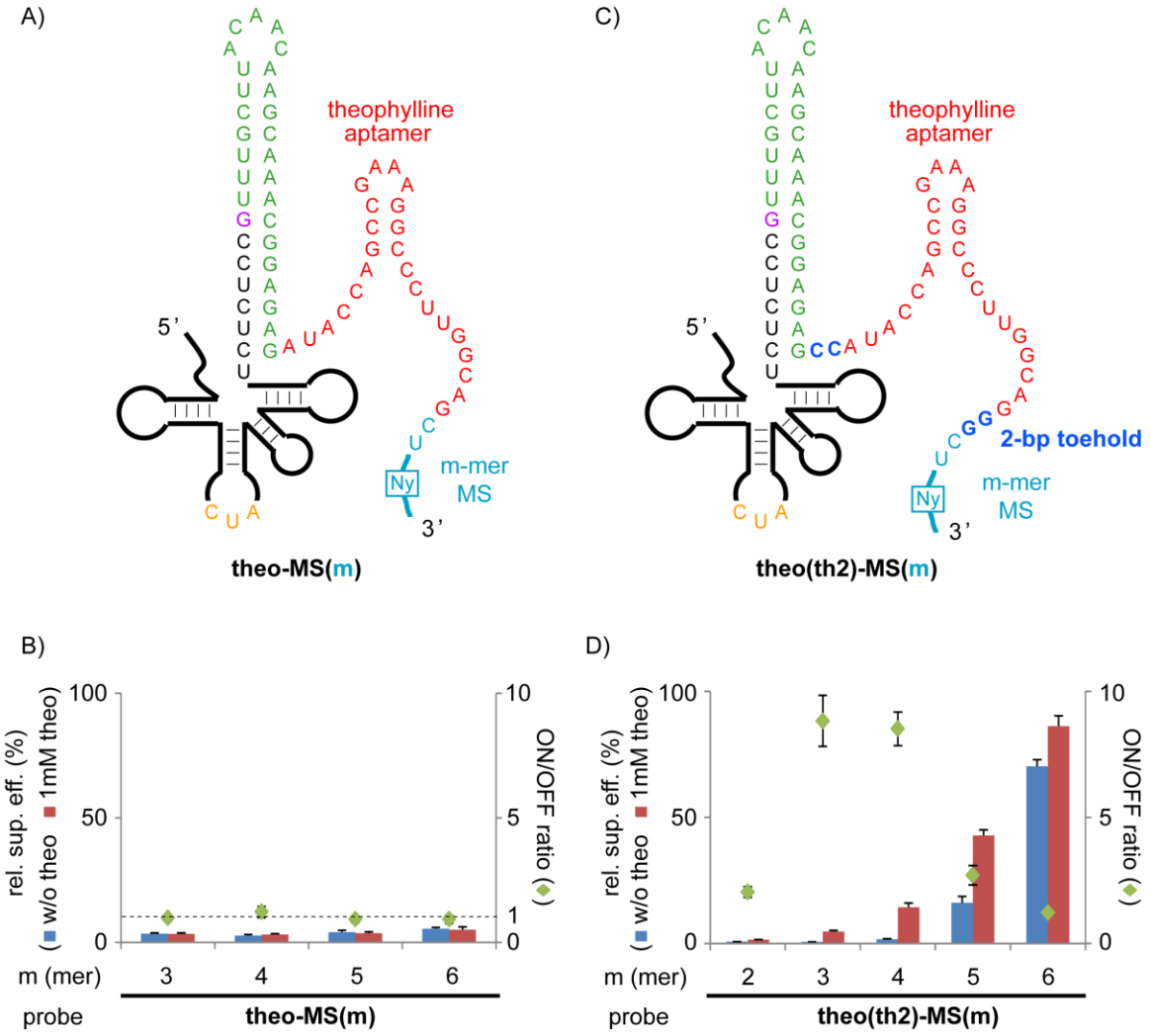


**Figure S1.** Other tRNA probe candidates for detecting theophylline. (A) and (C) Theophylline probes without a toehold (th) base pair (**theo-MS(m)**) and with a 2-bp th (**theo(th2)-MS(m)**), respectively, wherein m is the base length of the modulator sequence (MS). (B) and (D) The relative suppression efficiencies in the absence or presence of 1 mM theophylline (theo) and their ratios (ON/OFF) of **theo-MS(m)** and **theo(th2)-MS(m)**, respectively.

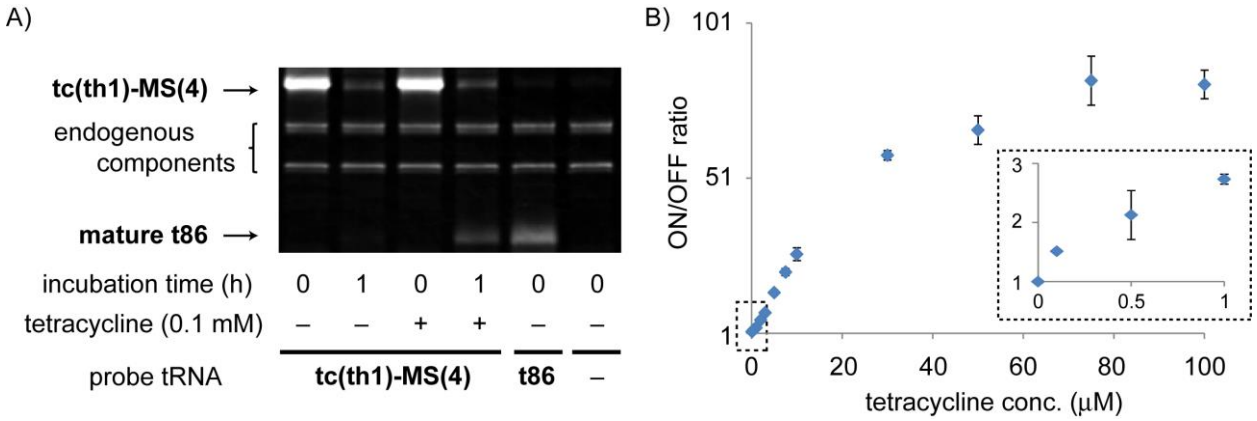


**Figure S2.** Characterization of the tetracycline probe, **tc(th1)-MS(4)**. (A) Gel analyses of **tc(th1)-MS(4)**, which was incubated in the absence or presence of 100 μM tetracycline for 0 or 1 h in WGE. 90% amounts of the probe were degraded in 1 h without the ligand, while about one-quarter underwent the 3' processing to become mature **t86** in the same time period when using 100 μM tetracycline. (B) The ON/OFF induction ratios of **tc(th1)-MS(4)** at various concentrations of tetracycline. (*Dotted box*) Expansion at low concentrations.

**References.**

(1) C. Kao, M. Zheng and S. Rüdisser, *RNA*, 1999, **5**, 1268.

(2) A. Ogawa, J. Tabuchi and Y. Doi, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 3724.

(3) A. Ogawa, *RNA*, 2011, **17**, 478.

(4) A. Ogawa and Y. Doi, *Org. Biomol. Chem.*, 2015, **13**, 1008.