Supporting Information

Genetic Screens and Selections for Small Molecules Based on a Synthetic Riboswitch that Activates Protein Translation

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General considerations

All plasmid manipulations were performed according to standard cloning techniques,[S1] and the sequences of all constructs have been verified by DNA sequencing at the NSF-supported Center for Fundamental and Applied Molecular Evolution at Emory University. Purification of plasmid DNA, PCR products, and enzyme digestions was performed using kits from Qiagen (Chatsworth, CA). Caffeine, theophylline, 3-methylxanthine, and *o*-nitrophenyl-β-D-galactopyranoside were purchased from Sigma. IPTG and X-gal were purchased from US Biological. Synthetic oligonucleotides were purchased from IDT.

Plasmid construction

All constructs are derived from the previously described plasmid pLacZU1hpII, which comprises a weak IS10 promoter, followed by a ribosome-binding site, and a gene encoding a fusion between a fragment of the bacterial IS10 transposase and the *lac*Z gene.[S2] Expression of this plasmid in *E. coli* provides the "no aptamer" control in Figure 1C.

Plasmid pSKD177.2 (wild-type aptamer)

The following strategy was used to introduce the mTCT8-4 aptamer into the 5'-UTR of the IS10-*lac*Z fusion gene in the pLacZU1hpII plasmid.[S2] pLacZU1hpII was used as a template for a PCR reaction with the forward primer SKD-057 and the reverse primer SKD-056. The PCR product was gel purified, digested with *Kpn*I and *Hind*III, gel purified, and cloned into the *Kpn*I and *Hind*III sites of pLacZU1hpII as described.[S2] The *Kpn*I site is located immediately 5' of the aptamer sequence and a 5bp spacer (*TATAA*) was included immediately 3' to the aptamer sequence before the ribosome-binding site of the IS10-*lac*Z gene.

SKD-057
5'–
CCCGGTACC**GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACC***TATAA*<u>AGACAACAAGATGTGCGAACTCG</u>
–3'
(**mTCT8-4 aptamer**, *5bp spacer*, <u>gene specific sequence</u>)

SKD-056
5′– CGACGGGATCGATCCCCCC –3′

Plasmid pSKD185.1 (C27A mutation in the aptamer sequence)

The mutation C27A (this the 27th nucleotide of the mTCT8-4 sequence as reported in Jenison, R. D.; Gill, S. C.; Pardi, A.; Polisky, B., *Science* **1994,** *263*, 1425-1429, it has alternatively been referred to as C22A in Soukup, G. A.; Emilsson, G. A.; Breaker, R. R., *J. Mol. Biol.* **2000,** *298*, 623-632.) was prepared using the QuikChange method (Stratagene) using pSKD177.2 as the template and primers SKD-065 and SKD-066.

SKD-065
5′– CAGCATCGTCTTGATGCCATTGGCAGCACCTATAAAG –3′
(C to A mutation in mTCT8-4 aptamer)

SKD-066
5′– CTTTATAGGTGCTGCCAATGGCATCAAGACGATGCTG –3′

Plasmid pSKD314 (chloramphenicol selectable plasmid)

A cassette mutagenesis strategy was used to generate pSKD314. The chloramphenicol acetyl transferase (*cat*) gene was cloned from pBAD33[S3] using PCR with the forward primer SKD-099 and the reverse primer SKD-094, to give product A. A separate PCR product (B) was generated using pSKD177.2 as a template with the forward primer SKD-071 and the reverse primer SKD-100. PCR products A and B contain an overlapping region and were mixed and amplified using the forward primer SKD-071 and the reverse primer SKD-094 to give PCR product C. PCR product C was digested with *Kpn*I and *Sac*I, gel purified, and cloned into the *Kpn*I and *Sac*I sites of pLacZU1hpII to afford plasmid pSKD305.1. A derivative of the Ptac1 promoter[S4] lacking the *lac* operator sequence was engineered in place of the IS10 promoter in pSKD305.1 by first generating a PCR product (D) using pSKD305.1 as a template with the forward primer SKD-125 and the reverse primer SKD-098. PCR product D was digested with *Spe*I and *Sac*I, gel purified, and cloned into the *Xba*I and *Sac*I sites of pUC18, forming plasmid pSKD314. pSKD314 thus comprised the *cat* gene fused in-frame to the 61st amino acid of IS10 through a 7 amino acid linker (Trp-Pro-Gly-Ser-Pro-Ala-Ser) along with the mTCT8-4 aptamer in the 5'-UTR of the gene fusion under the control of the Ptac1 derivative promoter.

SKD-099
5′– **GGCCTGGGTCCCCTGCTAGC**GAGAAAAAAATCACTGGATATACCACCGTTG –3′
(**7 amino acid linker,** gene specific segment)

SKD-094

5′ – GGCGCATGAGCTCTTACGCCCCGCCCTGCCACTCATCG –3′
(gene specific segment)

SKD-071
5′– CGGCCCGGCCATAAACTGCCAGGAATTAATTTAC –3′

SKD-100
5′– **GCTAGCAGGGGACCCAGGCCA**GATTCGTTTGATGTTATGTTTTGTTCTCGC –3′
(**7 amino acid linker,** gene specific segment)

SKD-125
5′–
TCAATCACTAGT**GAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGG**GCTAACAAGTCTAGCGAACCGCAC
–3′
(**Ptac1-derived promoter,** gene specific segment, *mutation eliminates SpeI site*)

SKD-098
5′ GTTAAATTGCCAACGCTTATTACCCAGCTCGATGC 3′

Plasmid pSKD441.1 (C27A mutation in aptamer of chloramphenicol selectable plasmid)

A cassette mutagenesis strategy was used to make plasmid pSKD441.1. A PCR product (A) was made using pSKD314 as a template with forward primer JPG007 and reverse primer SKD66. A separate PCR product (B) was formed using pSKD314 as a template with forward primer SKD65 and reverse primer JPG008. PCR products A and B contain an overlap, were mixed, and amplified using primers JPG007 and JPG008, forming PCR product C. PCR product C was digested with *Kpn*I and *Sac*I and cloned into these sites in plasmid pSKD314 forming plasmid pSKD441.1.

JPG-007
5′– GCGATTAAGTTGGGTAACGCCAGGG -3′

JPG-008
5′- GTATGTTGTGTGGAATTGTGAGCGG- 3′

Plasmid pSKD345.1 (transcriptional fusion)

The transcriptional fusion contained 3 stop codons after the 61st amino acid of the IS10 transposase in plasmid pSKD177.2 followed by a 28 bp pair spacer and then a ribosome binding site and *lac*Z gene cloned from pRS415.[S5] To make this construct, a PCR product (E) was generated using pSKD177.2 as a template with the forward primer SKD-071 and the reverse primer SKD-134. A second PCR product (F) was made using plasmid pRS415 as a template with the forward primer SKD-133 and the reverse primer SKD-098. PCR products E and F overlap, and they were mixed and amplified using primer SKD-071 and primer SKD-098, to give

PCR product G. PCR product G was gel purified, digested with *Kpn*I and *Sac*I, gel purified, and cloned into the *Kpn*I and *Sac*I sites in pLacZU1hpII, to give pSKD345.1.

SKD-133
5′- **CTGACTCCTCGAGTATAAAG**ACAACAAGATGACCATGATTACGGATTCACTGGCCGTC –3′
(overlapping region, gene specific segment)

SKD-134
5′– **CTTTATACTCGAGGAGTCAG**AGATCTCAGTTTATTATTAGATTCGTTTGATGTTATGTTTTGTTCTCGC –3′
(overlapping region, gene specific segment)

Growth curves of *E. coli* TOP10 cells

Three colonies of *E. coli* TOP10 cells harboring the wild-type synthetic riboswitch plasmid (SKD177.2) from an LB/agar plate containing ampicillin (50 μg/mL) were grown overnight at 37 °C with shaking in culture tubes containing LB media (5 mL) supplemented with ampicillin (50 μg/mL). 5 μL of the overnight culture was used to inoculate 1.5 mL of LB media in a 24-well plate (Costar) supplemented with ampicillin (50 μg/mL) and if necessary, appropriate concentrations of theophylline or caffeine. Plates were shaken at 215 rpm at 37 °C. The OD_{600} readings for each well were recorded using a plate-reading spectrophotometer (BioTek) hourly for seven hours, along with a final 24-hour reading. All growth studies were conducted in triplicate, and data is presented as the mean ± s.e.m.

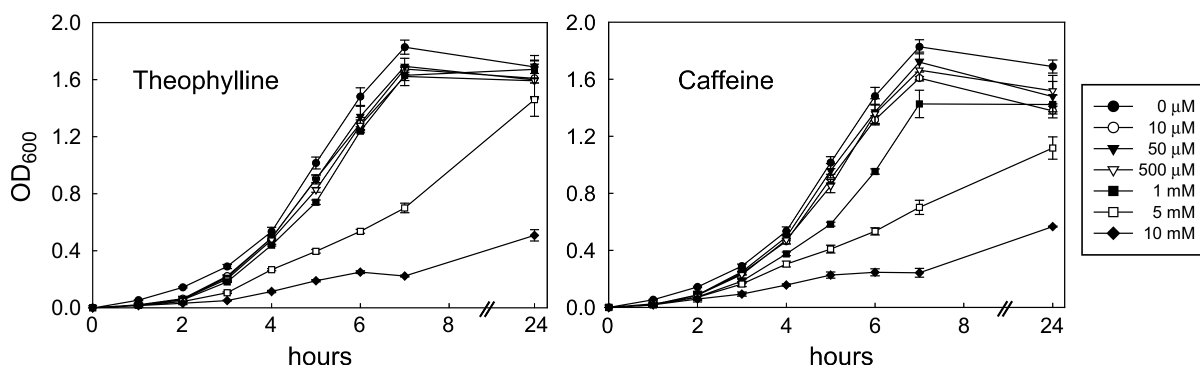

Figure S1. Growth curves for *E. coli* in the presence of varying concentrations of theophylline or caffeine.

[S1] Sambrook, J.; Russell, D. W., *Molecular Cloning : Laboratory Manual*. 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y., 2001
[S2] Jain, C.; Belasco, J. G., *Meth. Enzym.* **2000,** *318*, 309-332.
[S3] Guzman, L. M.; Belin, D.; Carson, M. J.; Beckwith, J., *J. Bacteriol.* **1995,** *177*, 4121-4130.
[S4] de Boer, H. A.; Comstock, L. J.; Vasser, M., *Proc. Natl. Acad. Sci. USA* **1983,** *80*, 21-25.
[S5] Simons, R. W.; Houman, F.; Kleckner, N., *Gene* **1987,** *53*, 85-96.