Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information Available

Cell-like Systems with Riboswitch Controlled Gene Expression

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Electronic Supplementary Material (ESI) for Chemical Communications

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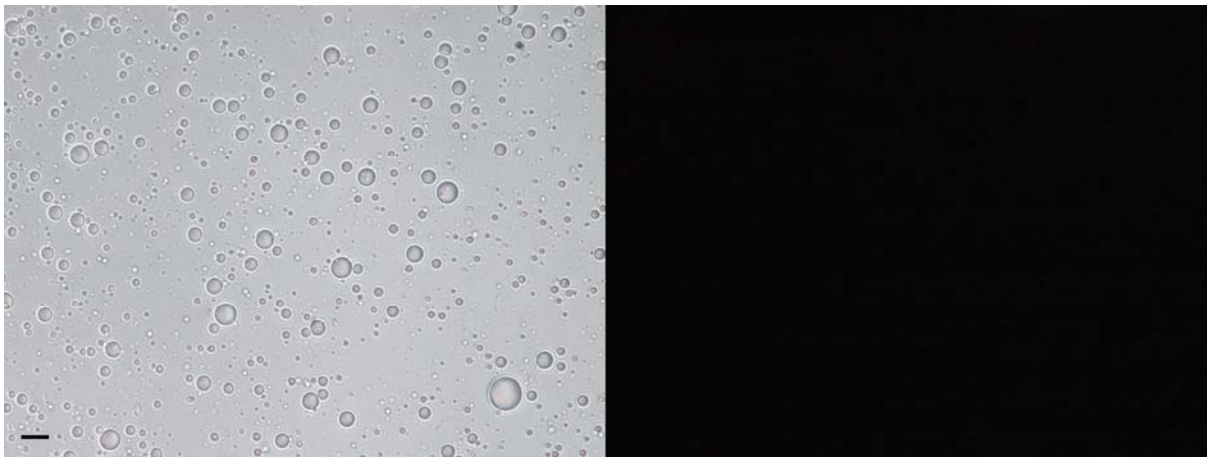

Figure S1. Control emulsion reaction. The theophylline riboswitch was encapsulated in w/o emulsion droplets with transcription-translation machinery provided by the PURE system. No theophylline was present. Bright field (left) and epifluorescence (right) images are shown. The black bar in the lower left corner of the bright field image (left) represents 10 μm.

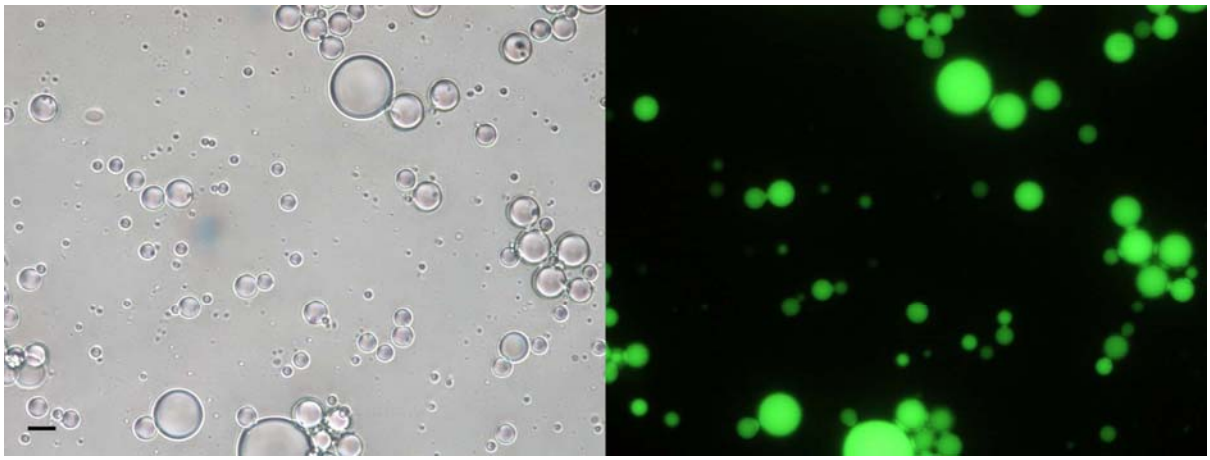

Figure S2. YPet emulsion expression. Robust expression of YPet with the PURE system inside of w/o emulsion droplets was achieved with a strong ribosome binding site. Here the strong ribosome binding site was provided by the adenine riboswitch construct. Bright field (left) and epifluorescence (right) images are shown. The black bar in the lower left corner of the bright field image (left) represents 10 μm.

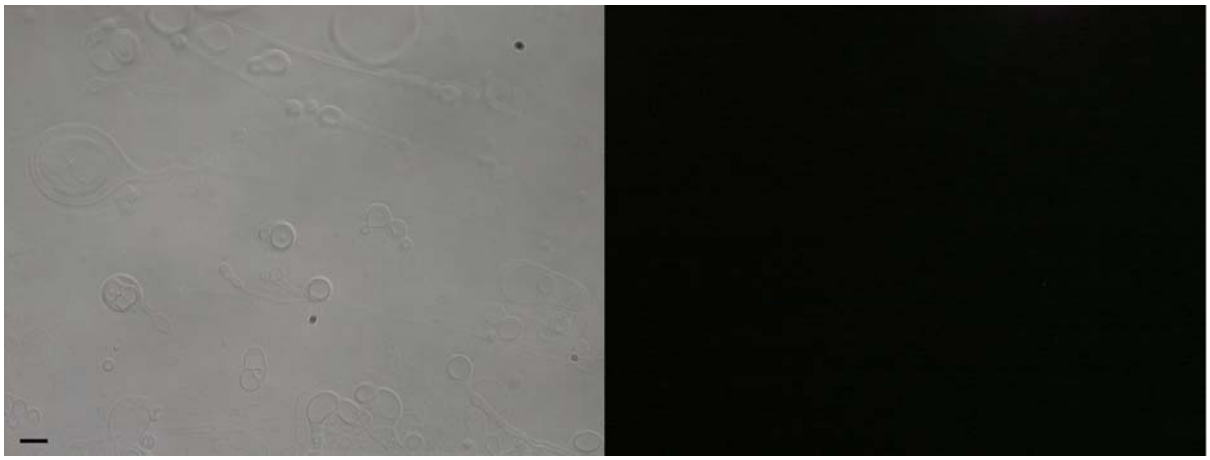

Figure S3. Control vesicle reaction. The theophylline riboswitch was encapsulated within phospholipid vesicles with transcription-translation machinery provided by the PURE system. No theophylline was present. Bright field (left) and epifluorescence (right) images are shown.

Methods

Materials. All genetic constructs were synthesized by Genscript. Unless otherwise indicated, all chemicals and plasmid purification kits were from Sigma-Aldrich. The *E. coli* cell lysate transcription-translation system (S30 T7 High Yield Protein Expression System) was from Promega, and the PURE system (PURExpress *in vitro* protein synthesis kit) was purchased from New England BioLabs. Lipids were either from Genzyme (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)), Sigma-Aldrich (cholesterol), or NOF-Europe (N-(carbonyl-methoxypolyethyleneglycol 5000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE-PEG 5000)).

***In vitro* transcription-translation with the PURE system.** The reactions were assembled on ice. All DNA templates were phenol-chloroform extracted and ethanol precipitated prior to use. 17.5 µL of the kit components were mixed with 250 ng DNA, 20 units RiboLock RNase Inhibitor (Fermentas), and RNase free Milli-Q water in a final volume of 25.5 µL. If adenine or theophylline was included, they were added to 1 µM and 500 µM, respectively. The entire reaction volume was then placed in a quartz micro cell cuvette (Hellma). Fluorescence measurements were with a PTI QuantaMaster 40 UV Vis Spectrofluorometer and the temperature was held at 37 °C with a Peltier temperature controlled single sample holder. Excitation and emission were at 517 nm and 530 nm, respectively. Emission was recorded every minute for 6 hours. Data were subsequently normalized by setting the initial emission to one. At least three sets of data were acquired for each riboswitch construct and averaged. The standard deviation for each data point was less than 40%.

***In vitro* transcription-translation with an *E. coli* S30 lysate.** The reactions were assembled directly in Nunc 96 well plates on ice. All DNA templates were phenol-chloroform extracted and ethanol precipitated prior to use. 38 µL of Promega S30 kit components (20 µL of premix factors plus 18 µL of *E. coli* S30 lysate), 40 units RiboLock RNase Inhibitor (Fermentas), 10 nM plasmid DNA, 8 mM magnesium acetate were mixed together, and RNase free Milli-Q water was added to bring to a final volume of 50 µL. If adenine or theophylline was included, they were added to 1 µM and 500 µM, respectively. Fluorescence emission was collected with a Tecan infinite 200 multiplate reader at 37 °C. The excitation wavelength was 510 nm, and the emission wavelength was 540 nm. Data were acquired every minute for 6 hours. The data were normalized as described above for "*In vitro* transcription-translation with the PURE system."

Water-in-oil emulsion reactions. Water-in-oil emulsions were prepared essentially as described by Davidson et al.[1] Briefly, 475 µL mineral oil, 22.5 µL Span-80, 2.5 µL Tween-80, and 0.25 µL Triton X-100 were stirred with a 9 mm Teflon stir bar in a 50 mL Falcon conical tube. 500 µL of this oil solution was then cooled on ice. Subsequently, 25.5 µL of chilled, aqueous solution containing all of the needed components for transcription-translation was added to 500 µL of the chilled oil phase and stirred for 3 min in an ice water bath. The aqueous phase contained all of the PURE system components plus 500 ng DNA template. If adenine or theophylline was included, the ligand was present at 1 µM and 5 mM, respectively. This concentration of theophylline was necessary due to the partitioning of theophylline to the oil phase. Reactions were initiated by incubating aliquots in

microcentrifuge tubes at 37 °C. Aliquots were visualized either with a Nikon Eclipse 90i or a Zeiss Observer Z1 microscope with FITC filters.

Vesicle Reactions. Vesicles were prepared essentially as described by Yomo and colleagues[2] with a few modifications. Briefly, 12 µmol of lipids consisting of 58:39:3 POPC:cholesterol:DSPE–PEG 5000 in chloroform were subjected to rotary evaporation in a pear-shaped flask with a Buchi Rotavapor R-210 and a Buchi Vacuum Pump V-700. The resulting thin lipid film was hydrated with 1 mL of deionized water, vortexed for 20 seconds, and then transferred to a 2 mL microcentrifuge tube. This lipid dispersion was homogenized for one minute with an IKA T 10 basic ULTRA-TURRAX disperser equipped with a 5 mm diameter dispersing tool and subsequently extruded through polycarbonate filters with 0.4 µm diameter pores (Whatman) using an Avanti Mini-Extruder (Avanti Polar Lipids, Inc.). 40 µL aliquots were frozen with liquid nitrogen and lyophilized overnight with a CentriVap Centrifugal Vacuum Concentrator (Labconco). Lyophilized liposomes were stored at -20 °C.

The following components were mixed on ice: 17.5 µL of PURE system kit components, 20 units RiboLock RNase Inhibitor (Fermentas), and 500 ng of DNA that was previously chloroform extracted and ethanol precipitated. If theophylline was included, it was added to 5 mM. The final volume of this reaction mix was 22.1 µL. 10 µL of this reaction mix was then used to hydrate an aliquot of lyophilized liposomes. The hydrated liposomes were incubated on ice for 2.5 h. Finally, the vesicle solution was diluted 20-fold with 50 mM Tris-HCl, 50 mM NaCl, pH 7.4 containing 10 mg/mL Proteinase K. For samples that were used to test the ability of the encapsulated riboswitch to sense extravesicular molecules, the samples were prepared without theophylline and then theophylline was added to 5 mM during the 20-fold dilution step. Samples were incubated at 37 °C in 0.2 mL microcentrifuge tubes. Aliquots were visualized with a Nikon Eclipse 90i microscope or a Zeiss Observer Z1 microscope with FITC filters.

Constructs used in this study. In the constructs shown below the underlined sequences represent transcriptional promoters, blue sequences represent riboswitches, normal black text indicates a sequence that encodes A206K YPet, bold type indicates an additional translational stop sequence (Registry of Standard Biological Parts code: BBa_B0042), red text represents the transcriptional terminator (BBa_B0011), and the ribosome binding site is shown in larger font. Finally, sequences with no intended function other than as a spacer are shown in italics. The theophylline riboswitch was deposited into the Registry of Standard Biological Parts (BBa_J89000).

Theophylline riboswitch 12.1[3] with a T7 promoter and A206K YPet

ATTTAATACGACTCACTATAGGGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACCCTGCTAAGGTAACAACA
AGATGGTGTCCAAAGGCGAAGAACTGTTTACCGGTGTGGTTCCGATTCTGGTGGAACTGGATGGCGACGTTAACGG
TCATAAATTTAGTGTGTCCGGCGAAGGTGAAGGCGATGCGACCTATGGCAAACTGACGCTGAAACTGCTGTGCACC
ACCGGTAAACTGCCGGTCCCGTGGCCGACCCTGGTGACCACGCTGGGTTATGGCGTGCAGTGTTTCGCGCGCTACC
GGACCACATGAAACAACACGATTTCTTTAAAAGTGCCATGCCGGAAGGCTATGTTCAGGAACGTACCATCTTTTTC
AAAGATGACGGTAACTACAAAACCCGCGCGGAAGTTAAATTTGAAGGCGATACGCTGGTCAACCGTATTGAACTGA
AAGGTATCGACTTCAAAGAAGATGGCAATATTCTGGGTCATAAACTGGAATATAACTACAATAGCCACAACGTGTA
TATTACCGCGGATAAACAGAAAAACGGCATCAAAGCCAACTTCAAAATCCGCCATAACATCGAAGACGGCGGTGTT
CAACTGGCCGATCACTACCAGCAAAACACCCCGATTGGTGATGGTCCGGTCCTGCTGCCGGATAATCATTATCTGT
CATACCAGTCGAAACTGTTTAAAGACCCGAATGAAAAACGTGATCACATGGTGCTGCTGGAATTTCTGACCGCGGC
CGGCATTACGGAGGGTATGAACGAACTGTATAAATGATAA**TTAGTTAGTTAG***CAGATCCGGCTGCTAACAAAGCCC*
*GAAAGGAAGCTGAGTTGGCTGCTGCCACCGTAGCAATAA*AGAGAATATAAAAAGCCAGATTATTAATCCGGCTTTT
TTGTTATTT

Theophylline riboswitch 12.1[3] with a *tac* promoter and A206K YPet

*GTTGACAATTAATCATCGGCTCG*TATAAT*GTGTGGCC*GGTGATACCAGCATCGTCTTGATGCCCTTGGCAGCACCC
TGCTAAGGTAACAACAAGATGGTGTCCAAAGGCGAAGAACTGTTTACCGGTGTGGTTCCGATTCTGGTGGAACTGG
ATGGCGACGTTAACGGTCATAAATTTAGTGTGTCCGGCGAAGGTGAAGGCGATGCGACCTATGGCAAACTGACGCT
GAAACTGCTGTGCACCACCGGTAAACTGCCGGTCCCGTGGCCGACCCTGGTGACCACGCTGGGTTATGGCGTGCAG
TGTTTCGCGCGCTACCCGGACCACATGAAACAACACGATTTCTTTAAAAGTGCCATGCCGGAAGGCTATGTTCAGG
AACGTACCATCTTTTTCAAAGATGACGGTAACTACAAAACCCGCGCGGAAGTTAAATTTGAAGGCGATACGCTGGT
CAACCGTATTGAACTGAAAGGTATCGACTTCAAAGAAGATGGCAATATTCTGGGTCATAAACTGGAATATAACTAC
AATAGCCACAACGTGTATATTACCGCGGATAAACAGAAAAACGGCATCAAAGCCAACTTCAAAATCCGCCATAACA
TCGAAGACGGCGGTGTTCAACTGGCCGATCACTACCAGCAAAACACCCCGATTGGTGATGGTCCGGTCCTGCTGCC
GGATAATCATTATCTGTCATACCAGTCGAAACTGTTTAAAGACCCGAATGAAAAACGTGATCACATGGTGCTGCTG
GAATTTCTGACCGCGGCCGGCATTACGGAGGGTATGAACGAACTGTATAAATGATAA**TTAGTTAGTTAG***CAGATCC*
*GGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGTAGCAATAA*AGAGAATATAAAAAGCCAGA
TTATTAATCCGGCTTTTTTGTTATTT

***B. subtilis* adenine riboswitch[4] with a T7 promoter and A206K YPet**

ATTTAATACGACTCACTATAGATTATCACTTGTATAACCTCAATAATATGGTTTGAGGGTGTCTACCAGGAACCGT
AAAATCCTGATTACAAAATTTTGTTTATGACATTTTTTGTAATCAGGATTTTTTTT*ATTTATCAAAACATTTAAGT*
*AA*AGGAGT*CTCGAG*ATGGTGTCCAAAGGCGAAGAACTGTTTACCGGTGTGGTTCCGATTCTGGTGGAACTGGATGG
CGACGTTAACGGTCATAAATTTAGTGTGTCCGGCGAAGGTGAAGGCGATGCGACCTATGGCAAACTGACGCTGAAA
CTGCTGTGCACCACCGGTAAACTGCCGGTCCCGTGGCCGACCCTGGTGACCACGCTGGGTTATGGCGTGCAGTGTT
TCGCGCGCTACCCGGACCACATGAAACAACACGATTTCTTTAAAAGTGCCATGCCGGAAGGCTATGTTCAGGAACG
TACCATCTTTTTCAAAGATGACGGTAACTACAAAACCCGCGCGGAAGTTAAATTTGAAGGCGATACGCTGGTCAAC
CGTATTGAACTGAAAGGTATCGACTTCAAAGAAGATGGCAATATTCTGGGTCATAAACTGGAATATAACTACAATA
GCCACAACGTGTATATTACCGCGGATAAACAGAAAAACGGCATCAAAGCCAACTTCAAAATCCGCCATAACATCGA
AGACGGCGGTGTTCAACTGGCCGATCACTACCAGCAAAACACCCCGATTGGTGATGGTCCGGTCCTGCTGCCGGAT
AATCATTATCTGTCATACCAGTCGAAACTGTTTAAAGACCCGAATGAAAAACGTGATCACATGGTGCTGCTGGAAT
TTCTGACCGCGGCCGGCATTACGGAGGGTATGAACGAACTGTATAAATGATAA*GCGGCCGC***TTAGTTAGTTAG***CAG*
*ATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGTAGCAATAA*AGAGAATATAAAAAGC
CAGATTATTAATCCGGCTTTTTTGTTATTT

***B. subtilis* adenine riboswitch[4] with a *tac* promoter and A206K YPet**

*GCTAGCGTTGACAATTAATCATCGGCTCG*TATAAT*GTGTGGCC*ATTATCACTTGTATAACCTCAATAATATGGTTT
GAGGGTGTCTACCAGGAACCGTAAAATCCTGATTACAAAATTTTGTTTATGACATTTTTTGTAATCAGGATTTTTT
TT*ATTTATCAAAACATTTAAGTAA*AGGAGTCTCGAGATGGTGTCCAAAGGCGAAGAACTGTTTACCGGTGTGGTTC
CGATTCTGGTGGAACTGGATGGCGACGTTAACGGTCATAAATTTAGTGTGTCCGGCGAAGGTGAAGGCGATGCGAC
CTATGGCAAACTGACGCTGAAACTGCTGTGCACCACCGGTAAACTGCCGGTCCCGTGGCCGACCCTGGTGACCACG
CTGGGTTATGGCGTGCAGTGTTTCGCGCGCTACCCGGACCACATGAAACAACACGATTTCTTTAAAAGTGCCATGC
CGGAAGGCTATGTTCAGGAACGTACCATCTTTTTCAAAGATGACGGTAACTACAAAACCCGCGCGGAAGTTAAATT
TGAAGGCGATACGCTGGTCAACCGTATTGAACTGAAAGGTATCGACTTCAAAGAAGATGGCAATATTCTGGGTCAT
AAACTGGAATATAACTACAATAGCCACAACGTGTATATTACCGCGGATAAACAGAAAAACGGCATCAAAGCCAACT
TCAAAATCCGCCATAACATCGAAGACGGCGGTGTTCAACTGGCCGATCACTACCAGCAAAACACCCCGATTGGTGA
TGGTCCGGTCCTGCTGCCGGATAATCATTATCTGTCATACCAGTCGAAACTGTTTAAAGACCCGAATGAAAAACGT
GATCACATGGTGCTGCTGGAATTTCTGACCGCGGCCGGCATTACGGAGGGTATGAACGAACTGTATAAATGATAAG
CGGCCGC**TTAGTTAGTTAG***CAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGTAG*
*CAATAA*AGAGAATATAAAAAGCCAGATTATTAATCCGGCTTTTTTGTTATTT

Supporting References

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