Supporting information

An Engineered Riboswitch as a Potential Gene-Regulatory Platform for Reducing Antibacterial Drug Resistance

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Materials and Methods. The plasmid manipulations were performed according to standard cloning techniques. Oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. The purifications of plasmid DNA, PCR products, and DNA after enzyme digestions were performed using kits obtained from Tiangen Biotech (Beijing) Co., Ltd. The Pfu DNA polymerase was purchased from Tiangen Biotech (Beijing) Co., Ltd. The AvrII and XhoI endonucleases, T4 polynucleotide kinase and T4 DNA ligase were purchased from TaKaRa Biotechnology Co., Ltd (Dalian China). Theophylline was obtained from Sigma.

Construction of plasmid pBV_{220}-Apt containing aptamer sequence. The modular plasmid, pBV_{220}, was constructed and used as a vector for the preparation of the riboswitch. The aptamer sequence was generated by PCR amplification using the appropriate oligonucleotide templates and primers, and then was cloned into two unique restriction sites (AvrII and XhoI), which located at three nucleotides downstream of the ampicillin-resistant (AMP^r) stop codon. The obtained cloned plasmid was transformed into *Escherichia coli* strain, DH5α, to obtain

pBV_{220}-Apt that was confirmed by subsequent sequencing (The Beijing Genomics Institute (BGI)). Confirmed plasmids were transformed into *Escherichia coli* strain, TOP10.

Growth curves of *E. coli* TOP10 cells. The colony of *E. coli* TOP10 cells harboring the plasmid PBV_{220}-Apt 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). 15 μL of the overnight culture was added into culture tubes containing 5 mL of LB media supplemented with ampicillin (50 μg/mL) and appropriate concentrations of theophylline, and the culture tubes were shaken at 37°C with a speed of 200 rpm. The optical density at 600 nm for each concentration of theophylline was recorded using a plate-reading spectrophotometer (BioTek) at 1 h intervals over 12 hours.

Assay for β-lactamase activity. Two separate colonies of *E. coli* TOP10 cells harboring pBV_{220} and pBV_{220}-Apt plasmids were respectively picked from an LB/agar plate without ampicillin and grown overnight at 37 °C in separate culture tubes containing LB media (5 mL). 15 μL of the overnight culture was added into culture tubes containing 5 mL of LB media supplemented with ampicillin (50 μg/mL) and appropriate concentrations of theophylline. Cells were grown at 37 °C with shaking to control the optical density at 600 nm in the range of 0.5 ~ 0.6, and the β-lactamase activity was assayed using a plate-reading spectrophotometer (BioTek) with nitrocefin as the chromogenic cephalosporin substrate. All assays were conducted in triplicate.

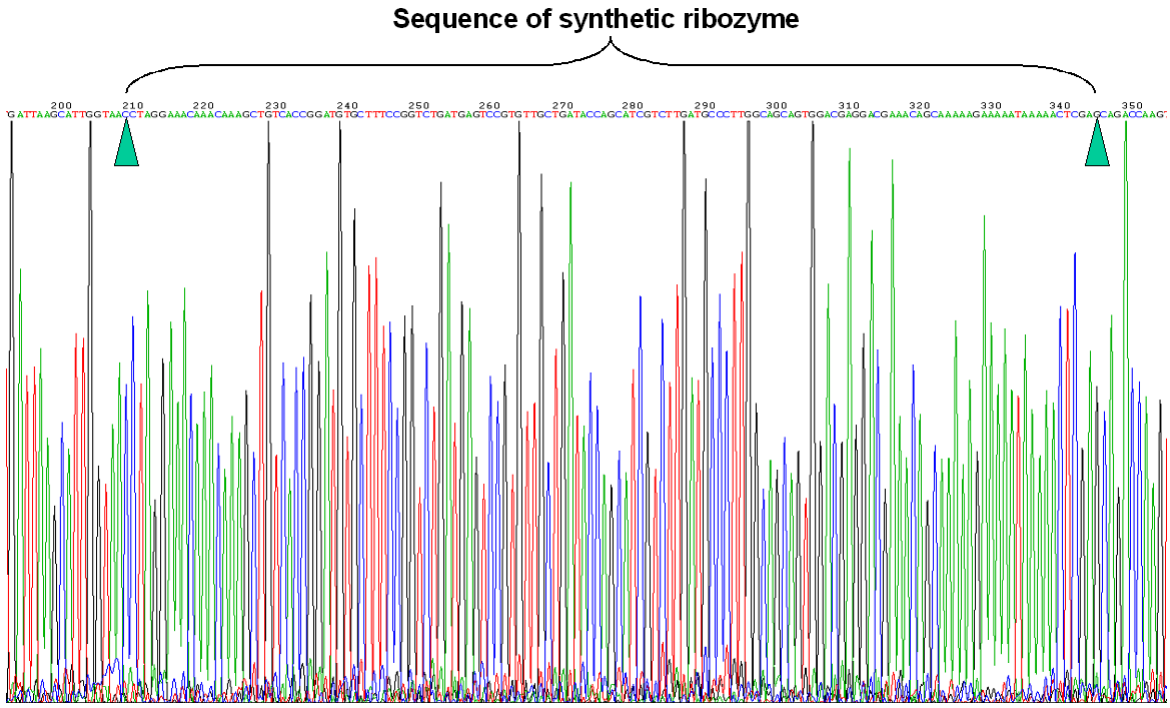

Figure S1. Sequencing result of synthetic ribozyme.