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Artificial caprolactam-specific riboswitch as an intracellular metabolite sensor

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ABSTRACT

Caprolactam is a monomer used for the synthesis of nylon-6, and a recombinant microbial strain for bio-based production of nylon-6 was recently developed. An intracellular biosensor for caprolactam can facilitate high-throughput metabolic engineering of recombinant microbial strains. Because of the mixed production of caprolactam and valerolactam in the recombinant strain, a caprolactam biosensor should be highly specific for caprolactam. However, a highly specific caprolactam sensor has not been reported. Here, we developed an artificial riboswitch that specifically responds to caprolactam. This riboswitch was prepared using a coupled *in vitro*-*in vivo* selection strategy with a heterogeneous pool of RNA aptamers obtained from *in vitro* selection to construct a riboswitch library used in *in vivo* selection. The caprolactam riboswitch successfully discriminated caprolactam from valerolactam. Moreover, the riboswitch was activated by 3.36-fold in the presence of 50 mM caprolactam. This riboswitch enabled caprolactam-dependent control of cell growth, which will be useful for improving caprolactam production and is a valuable tool for metabolic engineering.

KEYWORDS: Caprolactam; Riboswitch; SELEX; Metabolite biosensor; High-throughput screening

The bio-based manufacture of chemicals from renewable substrates using microorganisms is a promising approach that can substitute for chemical synthesis using petroleum-derived raw materials.[1] One important area in bio-based manufacture is the production of monomers.[2,3] Caprolactam (also known as ε-caprolactam) is a cyclic amide of caproic acid and is widely used to produce nylon-6 through ring-opening polymerization.[4] Because of the high economic value of caprolactam, there have been attempts to prepare microbes that produce caprolactam or its precursor.[5,6] However, the production strains require substantial improvement in the yield and titer of caprolactam or its precursor. Metabolic engineering of microbial strains often requires metabolic flux optimization and enzyme engineering, which can be achieved using a high-throughput screening system for a target chemical.[7,8]

Genetically encoded biosensors regulate gene expression according to the metabolite concentration in each cell. Biosensors offer unprecedented throughput for metabolite screening when combined with high-throughput methods such as fluorescence-activated cell sorting or artificial selection.[9–12] High specificity is one of the most important characteristics of a biosensor because the cytoplasm contains diverse molecules and some metabolic pathways produce byproducts with structures analogous to a target chemical. For example, a caprolactam-producing recombinant *Escherichia coli* strain co-produced valerolactam which differs by only a single carbon (i.e. caprolactam: C6, valerolactam: C5) because of the promiscuity of the pathway enzymes.[6]

Recently, a transcription factor (TF) -based lactam biosensor was developed.[13] Although this sensor responded to caprolactam, the sensor could not discriminate between caprolactam and valerolactam. However, it is not straightforward to engineer the specificity of a TF to make it respond to another target molecule. First, detailed information regarding the structure of the

ligand-binding pocket of the TF is necessary.[14,15] Second, even if the ligand-binding pocket is identified, engineering of the TF often requires high-throughput screening, which is limited by transformation efficiency.[15,16] Compared to protein-based sensors (e.g. TF), RNA-based sensors such as riboswitches have several advantages.[17] An RNA aptamer that specifically binds to a target chemical can be developed from large libraries because this process is performed *in vitro* and does not involve transformation.[18,19] Furthermore, an RNA-based sensor can reduce the unnecessary metabolic burden to a cell, as it directly interacts with the target molecule and does not rely on the expression of protein effectors.[20]

It remains challenging to develop an RNA-based sensor for a small molecule which has robust intracellular activity using a specified tight-binding aptamer through *in vitro* selection.[12,21] To overcome these limitations, we recently reported a coupled *in vitro–in vivo* selection process employing a heterogeneous aptamer population from *in vitro* selection to construct a riboswitch library for *in vivo* selection.[22] Utilization of the aptamer pool with diverse sequences increased the potential for discovering the riboswitch with *in vivo* functionality. Using this approach, naringenin-responsive riboswitches that function in *E. coli* cells were successfully discovered.

Here, we developed a caprolactam-specific artificial riboswitch for high-throughput screening and selection in microbial cells (Fig. 1). The coupled *in vitro–in vivo* selection strategy was utilized to develop the riboswitch. We validated caprolactam-dependent gene expression activation of the selected riboswitch. The specificity of the riboswitch to caprolactam was confirmed against other lactams and a caprolactam precursor, 6-aminocaproic acid. Additionally, the fold-activation of the riboswitch was measured and compared with that of the TF-based sensor which was reported in a previous study.[13] Finally, caprolactam-dependent control of cell growth was demonstrated using the riboswitch.

RESULTS

Development of artificial caprolactam riboswitch through coupled *in vitro-in vivo* selection

The development of a caprolactam-responsive artificial riboswitch requires an RNA aptamer that specifically binds to caprolactam. However, the tradeoff between binding affinity and the ability to rearrange the structure of an RNA aptamer is a well-known problem in developing a riboswitch.[21] Therefore, we employed a coupled *in vitro-in vivo* selection scheme[22] to successfully produce artificial riboswitches for small molecules (Fig. 1).

Following this scheme, an RNA aptamer library was enriched *in vitro* for binding to caprolactam. The initial RNA pool prepared by *in vitro* transcription of a double-stranded DNA template with a randomized sequence region was incubated with a solid matrix with caprolactam attached to the end of the linker. The RNA library contained a 60-nt-long randomized sequence region among which could serve as a caprolactam aptamer and the randomized region was flanked by two primer binding sites which were used to amplify the enriched RNA pool through reverse transcription and PCR (Fig. 2a). After washing, the bound RNA molecules were eluted and used to prepare the RNA pool for the next round by reverse transcription, PCR, and *in vitro* transcription. This selection cycle was repeated 10 times. At the 4th selection cycle, negative selection was carried out to eliminate possible false-positive RNAs that did not bind to caprolactam but bound to the solid matrix or its linker. The amount of RNA eluted in each step was measured to monitor the progress of *in vitro* selection (Fig. 2b). The *in vitro* selection was stopped after the 10th selection round and the eluted RNA fraction was 3.34%. The enriched RNA pool was amplified by reverse transcription and PCR and used directly to prepare a riboswitch library.

The riboswitch library was transformed to *E. coli* and selected *in vivo* to identify a functional riboswitch for controlling gene expression responding to caprolactam. Using *tetA* as a dual-selectable marker gene,[23,24] caprolactam-dependent gene expression activation was selected. Three negative selection steps (with 10 μM $NiCl_2$) with increasing concentration of caprolactam in the culture medium (0, 10, and 100 mg/L caprolactam) were followed by positive selection (with 50 μg/mL tetracycline) in the presence of a high concentration (1000 mg/L) of caprolactam. This whole sequence was repeated twice. After selection, single colonies were isolated and 10 colonies were tested for their ability to control gene expression in response to caprolactam. The gene expression level was quantified with or without caprolactam in the culture medium by measuring the fluorescence intensity from the superfolder green fluorescent protein. Colony #7 activated gene expression by 2.21-fold when 10 mM caprolactam was added to the culture medium (Fig. 2c). The DNA sequence of the caprolactam riboswitch from colony #7 was GGGAATTCGAGCTCCTGACAgcacaatttgcctacagcgttaagacacgaaaaagcacaattagacacttacatgtgtgg ATTCGAAGACGTCCAGCTGAactagccgga*aaggagcatctatgaaatctaacaatgcgctcatcgtcatc*. Capital letters indicate the primer binding sites, underlined letters indicate the randomized linker region, and italicized letters indicate the 5′-untranslated region followed by the first 30 nts of *tetA*.

Characterization of caprolactam riboswitch

We further characterized the important properties of the selected riboswitch for use in metabolic engineering. First, we evaluated the dose-response curve of the riboswitch. The fluorescence from the cell was measured with varying concentrations of caprolactam (0 – 50 mM) added to the culture medium. In the meantime, we measured the intracellular caprolactam concentration to validate that the riboswitch actually responds to the intracellular caprolactam (see Methods section). Indeed, the riboswitch activated the gene expression in response to the increasing

concentration of caprolactam (Fig. 3a, b). The operational range of the riboswitch was 3 – 50 mM of the extracellular caprolactam concentration (Fig. 3a) and 28.3 – 348.5 mM of the intracellular caprolactam (Fig. 3b). Notably, the riboswitch showed 3.36-fold-activation with 50 mM caprolactam, while the TF-based lactam sensor from the previous report showed 2.08-fold.[13] Thus, the caprolactam riboswitch had a higher fold-activation compared to the TF-based lactam sensor. Additionally, this riboswitch will be useful for future screening efforts because the best caprolactam producer at the moment produced only 79.60 μg/L (corresponds to 0.7 μM) caprolactam[6] and the riboswitch is responsive up to 50 mM.

Next, we investigated the specificity of the caprolactam riboswitch. A metabolite biosensor should be highly specific for a target molecule.[9] The engineered *E. coli* strain utilized 6-aminocaproic acid as a precursor and co-produced caprolactam and valerolactam.[6] Therefore, we tested the specificity of the caprolactam riboswitch against the precursor and two lactams with different carbon numbers (butyrolactam and valerolactam). First, 6-aminocaproic acid was not able to activate the riboswitch and even reduced the reporter gene expression slightly, showing 0.59-fold, when the chemical was added at 50 mM in the culture medium (data not shown). Next, we tested the specificity of the riboswitch against the lactams. We supplemented the lactams at 50 mM in the culture medium and measured the fluorescence. Further, we measured the intracellular concentration of the lactams because the difference of uptake efficiency of the chemicals could affect the response from the riboswitch. The riboswitch activated gene expression much higher when caprolactam was added as an inducer compared to the other chemicals although the intracellular concentrations of the chemicals were comparable (Fig. 3c). Therefore, the specificity of the riboswitch to caprolactam against the precursors and similar chemicals was confirmed.

We further tested the specificity by mimicking the producer strain. Since the engineered strain co-produced caprolactam and valerolactam, we measured the fluorescence from the cell cultured with varying ratios of caprolactam and valerolactam while maintaining their total concentration constant. The fluorescence increased linearly with the increasing percentage of caprolactam (Fig. 3d). Therefore, the riboswitch will be useful for screening caprolactam producers even with the promiscuous co-production of valerolactam.

Caprolactam-dependent control of cell growth using the caprolactam riboswitch

Finally, we demonstrated the ability of using the riboswitch as an artificial selection device. Because of its simple experimental setup and high throughput, growth-based selection is regarded as one of the best choices for the screening of metabolite production if a suitable selection system is provided.[7,24,25] Because the riboswitch regulates the expression of a selection marker, *tetA*, in response to caprolactam, we assumed that the riboswitch could be utilized as a selection device. When tetracycline was not added to the culture medium, the growth rate of *E. coli* transformed with the riboswitch decreased slightly with increasing caprolactam concentrations because of the toxicity of caprolactam (Fig. 4). In the presence of tetracycline, however, the growth rate at a high caprolactam concentration was higher than that with a low concentration. Interestingly, the selection cutoff concentration was modulated by changing the tetracycline concentration. With 80 μg/mL tetracycline, only *E. coli* cells without caprolactam were unable to grow. When more tetracycline was added to the medium (150 or 300 μg/mL), the minimal caprolactam concentration required for growth increased accordingly. Therefore, the selection cutoff level can be controlled simply by adjusting the tetracycline concentration depending on the productivity of the parental strain used to prepare the producer mutant library.

DISCUSSION

In this study, we developed an artificial caprolactam riboswitch for quantitatively detecting the intracellular concentration of caprolactam. Key parameters such as specificity, fold-activation, and linear range of detection were evaluated to assess whether the riboswitch was an advanced selection device compared to the TF-based sensor. Finally, we utilized the riboswitch for artificial selection. A caprolactam riboswitch was successfully developed using a coupled *in vitro-in vivo* selection strategy. The riboswitch specifically responded to caprolactam and effectively discriminated it from butyrolactam and valerolactam. The fold-activation of the riboswitch was 3.36-fold and the riboswitch was able to detect caprolactam up to 50 mM. Finally, the riboswitch enabled the control of cell growth depending on the caprolactam concentration, indicating the potential application of the riboswitch in artificial selection.

One of the significant improvements of the riboswitch as a caprolactam sensor compared to the TF-based sensor was its specificity for caprolactam. Valerolactam and caprolactam are co-produced because of the promiscuity of the pathway enzymes,[6] necessitating a metabolite sensor that specifically responds to caprolactam. The TF-based sensor could not distinguish between caprolactam and valerolactam,[13] while the two lactams were easily discernable using the riboswitch. The difference in specificity may be related to dissimilarity between sensor development strategies. The TF-based lactam sensor was discovered from nature by searching for a TF that responds to a molecule with a structure analogous to valerolactam. Therefore, the TF was more likely to respond to valerolactam rather than to caprolactam. Indeed, the fold-activation of the TF-based lactam sensor was higher for valerolactam than for caprolactam. In contrast, our strategy aimed to directly evolve an RNA aptamer that binds to caprolactam. As a result, the selected riboswitch responded more specifically to caprolactam than to valerolactam. We predicted that the development of a specific metabolite sensor could be streamlined by

employing the coupled *in vitro-in vivo* selection strategy for the riboswitch, considering that engineering of specific TF-based sensors often requires detailed information about the protein structure and substantial efforts to design and select mutant libraries.

In addition to its specificity, the caprolactam riboswitch showed promising properties as an intracellular metabolite sensor. The riboswitch activated gene expression by 3.36-fold at 50 mM caprolactam while the TF-based sensor from the previous report activated expression by 2.08-fold at the same concentration. Additionally, caprolactam-dependent control of cell growth was demonstrated using the riboswitch. The selection cutoff caprolactam concentration was easily modulated by adjusting the tetracycline concentration.

Although the riboswitch developed in this study will be a useful tool, the best titer from an engineered strain to date (0.7 μM) is much lower than the detection limit of the riboswitch and small improvement from this strain would not be easy to be detected. If necessary, the sensitivity of the riboswitch could be improved in several ways. For example, the number of an aptamer can be modulated to adjust the sensitivity.[26] Otherwise, a synthetic circuit for signal amplification could be utilized to improve the sensitivity of a riboswitch.[27]

In summary, we developed a specific caprolactam riboswitch useful for monitoring the intracellular concentration of caprolactam. The riboswitch developed through coupled *in vitro-in vivo* selection responded specifically to caprolactam. The riboswitch exhibited good sensor parameters such as a high fold-activation (3.36-fold) and an operational range of up to 50 mM. Furthermore, the capability of the riboswitch as a caprolactam selection device was demonstrated. This riboswitch is a valuable tool for directed evolution of enzymes and pathway optimization for the microbial production of caprolactam.

METHODS

Bacterial strains, plasmids, and reagents

Escherichia coli strains and plasmids used in this study are listed in Table S1. Oligonucleotides were synthesized by Cosmogenetech (Seoul, Korea) and are listed in Table S2. All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

***In vitro* selection of RNA aptamers for caprolactam**

The RNA aptamer for caprolactam was selected *in vitro* through the systematic evolution of ligands by exponential enrichment (SELEX).[28,29] The caprolactam-coupled solid matrix was prepared based on ECH Sepharose 4B (GE Healthcare Life Science, Little Chalfont, UK). Because this matrix can be coupled to a molecule with a primary amine group, we used L-(−)-α-amino-ε-caprolactam, which is differed from ε-caprolactam only by an additional primary amine group. The coupling reaction was carried out following the manufacturer's instruction. The pre-swollen matrix was washed with distilled water (pH 4.5), followed by washing with 0.5 M NaCl. This matrix was mixed with an equal volume of ligand solution (0.1 M in distilled water, pH 4.5). Next, *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride was added at a final concentration of 0.1 M. The reaction mixture was incubated overnight at room temperature. After the coupling reaction, the matrix was subjected to alternating washes using 0.1 M acetate buffer (pH 4.0, with 0.5 M NaCl) and 0.1 M Tris-HCl buffer (pH 8.0, with 0.5 M NaCl) three times. The coupled matrix was stored in 20% ethanol at 4°C until use.

Preparation of the RNA pool and selection of aptamers were performed following the protocol from a previous SELEX experiment.[22] A DNA template was prepared by PCR amplification of the SELEX_Template with SELEX_F and SELEX_R using Prime Taq DNA polymerase (GeNet Bio, Daejeon, Korea). The DNA template was transcribed by T7 RNA

polymerase (New England Biolabs, Ipswich, MA, USA) and treated with DNaseI (Takara Bio, Shiga, Japan). The transcribed RNA pool was purified by polyacrylamide gel electrophoresis. One milliliter of RNA solution (3 μM) in selection buffer (50 mM Tris-HCl, 100 mM KCl, pH 7.4) was heated at 95°C for 10 min and cooled at room temperature for 20 min. Next, $MgCl_2$ was added at a final concentration of 10 mM and the solution was further incubated at room temperature for 15 min. The RNA solution was mixed with 1 mL of the caprolactam-coupled matrix and incubated overnight at room temperature with gentle rotation. Negative selection was performed at round 4 using a non-coupled matrix to eliminate RNA molecules that bound to the matrix.

The RNA-matrix slurry was washed with 10 mL of washing buffer (50 mM Tris-HCl, 100 mM KCl, 10 mM $MgCl_2$, pH 7.4), and then 2 mL of elution buffer (25 mM Tris-HCl, 300 mM NaCl, 5 mM EDTA, 4 M urea, pH 7.4) was heated to 95°C and added to the matrix. After vigorous mixing by pipetting, the slurry was filtered, and the elution buffer was precipitated using ethanol. RNA was quantified using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan) at 260 nm.

The DNA pool for the next round of selection was prepared by reverse transcription-polymerase chain reaction (RT-PCR). cDNA of the eluted RNA was synthesized by SuperScriptIII (Invitrogen, Carlsbad, CA, USA) using SELEX_R as a primer. The cDNA was amplified by Q5 DNA polymerase (New England Biolabs) using SELEX_RT_T7 and SELEX_R as primers. The amplified DNA pool was used as a template for *in vitro* transcription. After round 4, the washed RNA was used to prepare the solution for round 5.

***In vivo* selection of caprolactam riboswitch**

The riboswitch library plasmid was cloned using BsaI sites in the backbone plasmid (pRibo_NC). The eluted RNA pool after SELEX round 10 was reverse-transcribed using the CapApt_N10_R primer. The cDNA was PCR amplified using CapApt_F and CapApt_N10_R as primers. The amplified DNA was digested by BsaI-HF (New England Biolabs) and ligated with the digested backbone plasmid using T4 DNA ligase (Takara Bio). The ligation mixture was precipitated by ethanol and transformed into MegaX DH10B $T1^R$ electrocompetent cells (Invitrogen). The cloned plasmid was extracted and transformed into *E. coli* W3110.

The *E. coli* library transformed with the riboswitch library was inoculated into 3 mL of M9 medium containing 34 μg/mL chloramphenicol (CM9) at an OD_{600} of 0.05. This medium also contained an appropriate concentration of caprolactam for cell adaptation. The cells were cultured for 8 h at 37°C with shaking (220 rpm) and all other culture in this study was conducted under the same conditions. The culture broth was diluted to 3 mL with fresh CM9 containing caprolactam at the same concentration and appropriate selection pressure at an OD_{600} of 0.01. This selection broth was cultured until the OD_{600} reached 0.5. Selection was performed as follows: negative (0 mg/L caprolactam), negative (10 mg/L caprolactam), negative (100 mg/L caprolactam), and positive (1000 mg/L caprolactam). Either 10 μM $NiCl_2$ or 50 μg/mL tetracycline was supplemented to the medium for negative or positive selection, respectively. The whole selection sequence was repeated twice. Ten colonies were isolated after the whole selection process on an LB agar plate containing chloramphenicol. The colonies were cultured in CM9 medium with or without 10 mM caprolactam. The OD_{600} and fluorescence of the culture broth were measured using a $VICTOR^3$ 1420 Multilabel Counter (PerkinElmer, Waltham, MA, USA). Fluorescence was detected using a 485-nm excitation filter and 535-nm emission filter with a 1-s measurement time. Specific fluorescence (S.F.) and fold-activation (F.A.) were

calculated with the following equations: S.F. = ($Fl._{culture}$ - $Fl._{background}$)/($OD_{600,\ culture}$ - $OD_{600,\ background}$), F.A. = $S.F._{+cap}/S.F._{-cap}$. The CM9 medium was used as a background for fluorescence and OD_{600} measurements. All cultures were performed in biological triplicate.

Characterization of caprolactam riboswitch

The *E. coli* W3110 cells with the caprolactam riboswitch were cultured overnight in 3 mL of CM9. The culture broth was diluted at a final OD_{600} of 0.05 into 3 mL of CM9 and cultivated for 8 h. Then, to evaluate the dose-response curve, the culture broth was inoculated into 6 mL of CM9 medium containing various concentrations of caprolactam (0, 1, 3, 10, 20, 30, 50 mM) at a final OD_{600} of 0.05. To investigate the specificity, 6-aminocaproic acid (50 mM), butyrolactam (50 mM), valerolactam (50 mM), or caprolactam (50 mM) was added to the culture medium. For the caprolactam-valerolactam mixture experiment, two lactams of different ratios were added to the culture medium while maintaining their total concentration at 70 mM. The culture broth was incubated for 12 h and the fluorescence and OD_{600} were measured using the VICTOR^3 to calculate the specific fluorescence and fold-activation. Remained cells were used for the metabolite extraction. All cultures were performed in biological triplicate.

Intracellular metabolite extraction

4×10^9 Cells were collected by filtration using a mixed cellulose ester membrane disk filter of pore size 0.22 μm (Millipore, Bedford, MA). Then, the pre-chilled isotonic solution was filtrated to wash media components. Next, the filter was immersed in 5 mL of chloroform/methanol/water (32:60:8, v/v/v) solution and vortexed. The solution was centrifuged at 8000 rpm for 10 min at 0 °C and 0.4 mL of the aqueous phase was transferred to 1.5 mL tube. Then the solution was fully dried using vacuum dryer (Hanil Science Industrial Co., Incheon, Korea) and kept on -80 °C until analyzed.

Intracellular metabolite sample preparation and derivatization

Caprolactam, butyrolactam, and valerolactam samples were dissolved in 200 μL of distilled water containing hexanol (Sigma Aldrich) as an internal standard. The samples were then centrifuged at 14,000 rpm for 10 min to pellet any undissolved residue, and the supernatant was transferred to a new glass insert for GC-MS injection.

GC-MS analysis of intracellular metabolites

Sample analysis was performed on a GC-MS system, GC 7890 coupled to an MSD 5977 (Agilent Technologies, Inc., Santa Clara, CA) equipped with an HP-5MS capillary column (30 m ×0.25 mm i.d.×0.25 μm; Agilent J&W Scientific). The injection volume was 1 μL and all samples were run in 1:10 split mode with an inlet temperature of 270 °C. Helium flow rate was set to 1 mL/min. The MS source temperature was maintained at 230 °C, and the MS quad temperature was held constant at 150 °C with electron energy of 70 eV. The oven temperature profile was 40 °C for 5 min; 3 °C/min to 160 °C; 20 °C/min to 300 °C; and held at 300 °C for 15 min. The selected ions monitored were 113 m/z for caprolactam, 85 m/z for butyrolactam and 99 m/z for valerolactam.

Tetracycline resistance assay

The *E. coli* W3110 cells with the riboswitch were cultured overnight in CM9. The culture broth was diluted to 3 mL of CM9 containing various concentrations of caprolactam (0, 10, 20, and 50 mM) and cultured for 6 h at a final OD_{600} of 0.05. The culture broth was diluted with 3 mL of CM9 containing different combinations of caprolactam concentrations (0, 10, 20, and 50 mM) and tetracycline concentrations (0, 80, 150, and 300 μg/mL) at a final OD_{600} of 0.05. Growth rates were evaluated by measuring the OD_{600} of the cultures. All cultures were performed in biological triplicate.

ASSOCIATED CONTENT

Supporting Information

(Table S1) *E. coli* Strains and plasmids used in this study

(Table S2) Oligonucleotides used in this study

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Author contributions

[1]S.J. and S.J. contributed equally to this work. S.J., S.J, D.-K. I., T.J.K., M.-K. O. and G.Y.J. designed the project. S.J., S.J. and D.-K. I. performed the experiments. S.J., S.J., D.-K. I., and G.Y.J. analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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FIGURE LEGENDS

Figure 1. Overall development strategy for the caprolactam riboswitch.

Figure 2. Development of the caprolactam riboswitch. (a) Preparation of the RNA library for SELEX. P_{T7}, T7 promoter; PBS, primer binding site; N_{60}, randomized sequence region of 60 nt (b) SELEX profile. Negative selection was performed at round 4 using the intact matrix. (c) Fold-activation of ten colonies after *in vivo* selection. Specific fluorescence with 10 mM caprolactam was normalized to that with no caprolactam.

Figure 3. Caprolactam-specific gene activation by the caprolactam riboswitch. (a, b) Dose-response curves of the riboswitch for caprolactam using extracellular or intracellular caprolactam concentration. (c) Specificity of the riboswitch to caprolactam against other lactams. C4, butyrolactam; C5, valerolactam; C6, caprolactam (d) Specificity of the riboswitch to caprolactam when the mixture of caprolactam and valerolactam was added to the culture medium. All experiments were performed in biological triplicate and the error bars indicate the standard deviations.

Figure 4. Caprolactam-dependent control of cell growth using the caprolactam riboswitch. The numbers in squares are averaged growth rates from biological triplicate.

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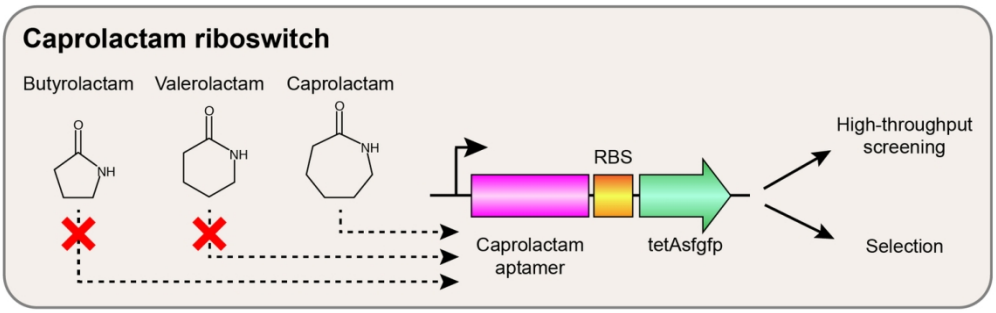

Graphical Table of Contents

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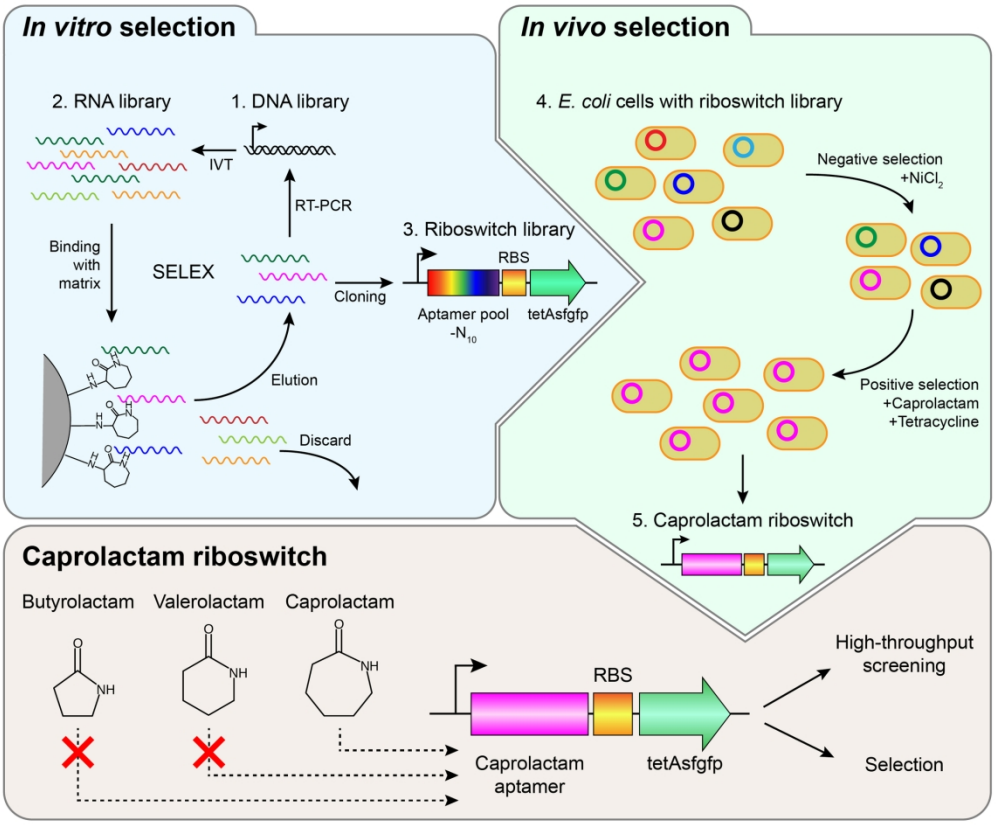

Figure 1

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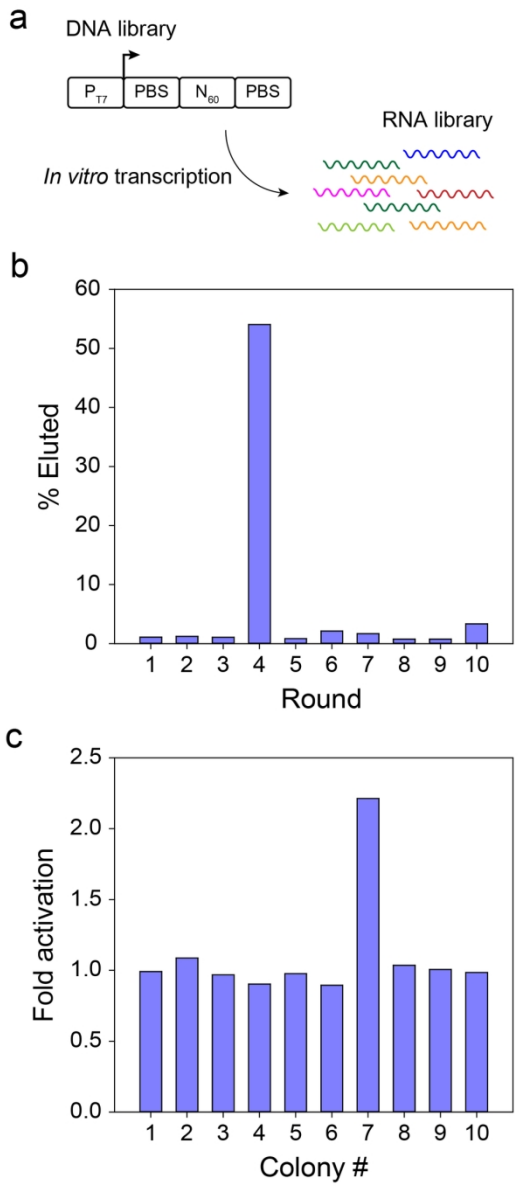

Figure 2

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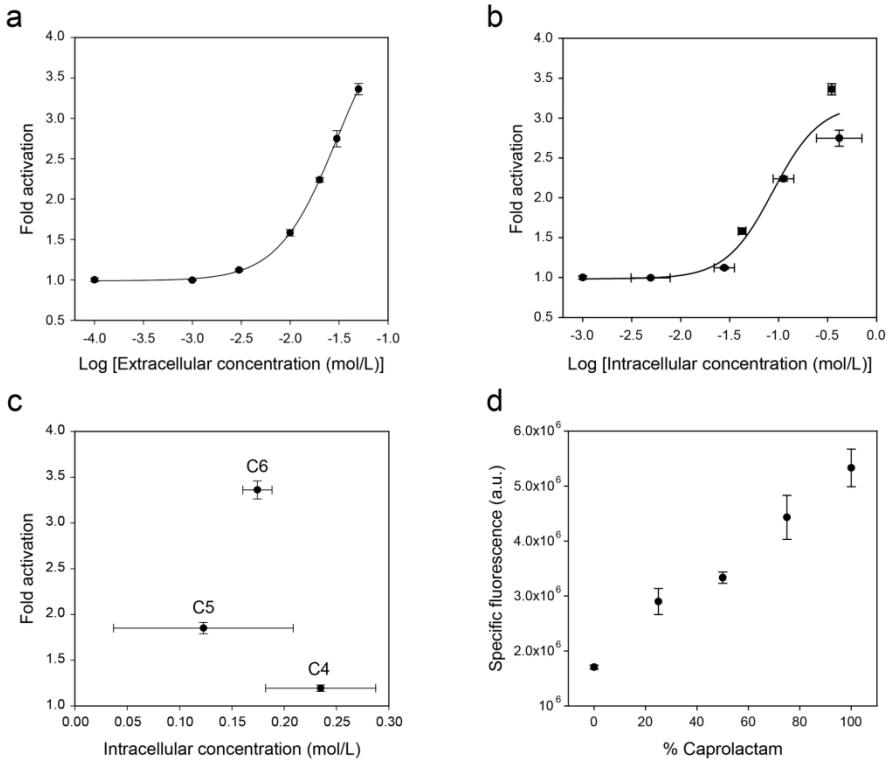

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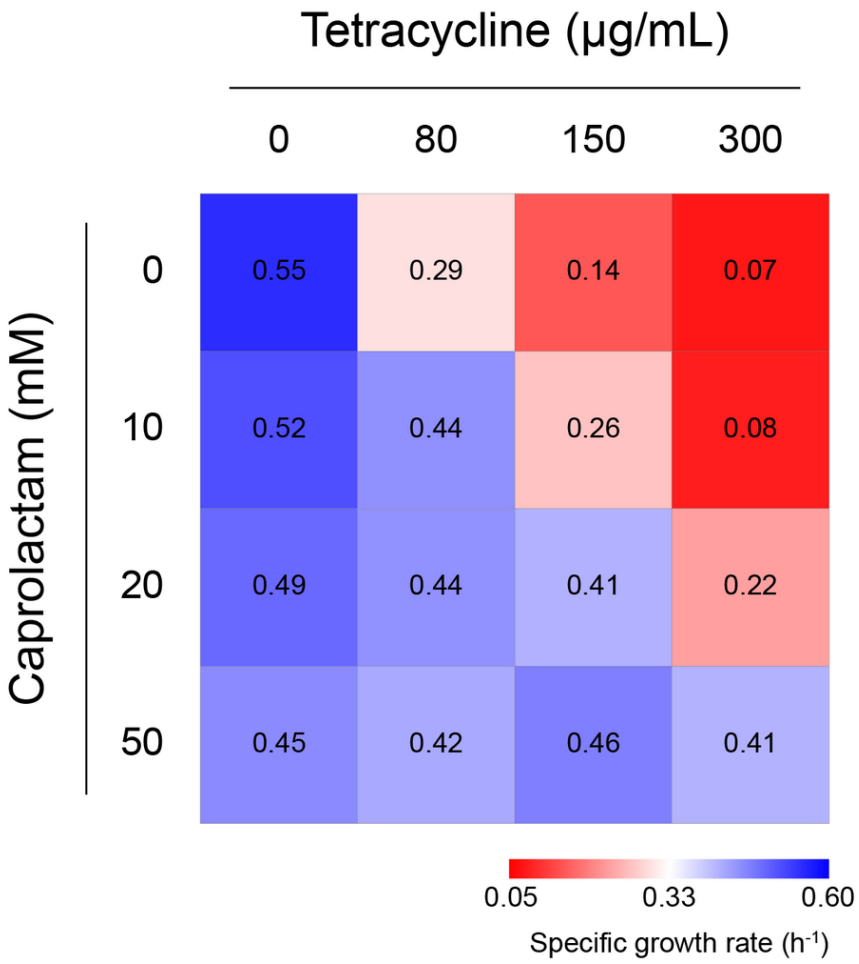

Figure 4

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