Supporting Information

Application of mRNA arrays for the production of mCherry reporter-protein arrays for quantitative gene expression analysis

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Supporting Methods

Preparation of DNA and mRNA capture surfaces

Two NHS-activated microarray slides (Nexterion H, Schott) were assembled in a sandwich arrangement so that the activated surfaces faced each other. 30 µl of either 1 mg/ml or 2 mg/ml streptavidin, in phosphate buffered saline pH 7.4 (PBS), were pipetted between the slides, as filling, to produce the DNA and mRNA capture surfaces, respectively. The assembly was incubated in a humidity chamber at 37°C for 30 minutes. To block any unreacted NHS functional groups, the slides were incubated in 50 ml 50 mM ethanolamine pH 8.5 in PBST (PBS containing 0.02% (v/v) Tween 20) at room temperature, with rocking, for 20 minutes. A standard wash and dry step was performed as follows: a wash with 50 ml PBST at room temperature, with rocking, for 5 minutes followed by a wash with 50 ml MilliQ H₂O at room temperature, with rocking, for 30 seconds and a final rinse with MilliQ H₂O. The slides were placed in a 50 ml Falcon tube and dried by centrifugation at 180 g, at room temperature for 30 seconds.

Construction of pET28-mCherry-SpyTagsa and pET28-SX-mCherry-SpyTagsa plasmids

A SpyTagsa scaffold (see Table S1 for details) was obtained from GeneArt (Thermo Fisher Scientific). This was cloned into the BglII and XhoI sites of pET28a (Merck) to generate plasmid pET28-SpyTagsa. Sequence encoding mCherry was amplified from p3-mCherry (a kind gift from the European Xenopus Resource Centre) using forward primer 5'-CGA GGC CAT ATG ATG GTG AGC AAG GGC GAG GA-3' and reverse primer 5'-GCTC CGG CTA GCC TTG TAC AGC TCG TCC ATG CCG-3'. This was cloned into the NdeI and NheI sites of pET28-SpyTagsa to generate pET28-mCherry-SpyTagsa (Table S1). A series of pET28-toehold X-mCherry-SpyTagsa toehold switch plasmids, based on the second-generation toehold switches 1, 3, 8 and 9 described in Green et al., 201413, were generated by homologous recombination30,31. Briefly, linear pET28-mCherry-SpyTagsa plasmid was generated using pET28-mCherry-SpyTagsa as the template, forward primer 5'-CAC CAC CAC CAC CAT ATG ATG GTG AGC-3' and reverse primer 5'-AGA TCT CGA TCC TCT ACG CCG GAC GCA TC-3'. Toehold switches, flanked
at the 5’ end by sequence with homology to pET28 and the T7 promoter, and at the 3’ end by
sequence with homology to His$_{6}$-mCherry, were generated by overlap extension PCR (see Table S2
for primer sequences). PCR products were purified using Nanosep centrifugal filter columns with a
10 kDa MWCO (Pall Corporation). 

\textit{Escherichia coli} DH5$\alpha$ cells were transformed with approximately
5 fmol of a mixture of a 1:10 molar ratio of linearized plasmid:toehold switch PCR product.

**Preparation of \textit{in vitro} transcription templates**

5’-biotinylated, 3’-Alexa647-labelled mCherry-SpyTag$_{sa}$ \textit{in vitro} transcription template (see Table S3)
was generated by PCR using the pET28-mCherry-SpyTag$_{sa}$ plasmid as the template, forward primer
5’-biotin-CGA GGC AGA TCT TAA TAC GAC TCA CTA TAG-3’ and reverse primer 5’-Alexa647-GCA TGC
ATC CCG GCC CGC GAC TAT CTT ACG CA-3’. 5’-biotinylated, 3’-Dy549-labelled mCherry-SpyTag$_{sa}$ and
toehold-mCherry-SpyTag$_{sa}$ \textit{in vitro} transcription templates (see Table S3) were generated by PCR
using either pET28-mCherry-SpyTag$_{sa}$ or the relevant pET28-toehold-mCherry-SpyTag$_{sa}$ plasmid as
the template, forward primer 5’-biotin-CGA GGC AGA TCT TAA TAC GAC TCA CTA TAG-3’ and reverse
primer 5’-Dy549-GCA TGC ATC CCG GCC CGC GAC TAT CTT ACG CA-3’. Unlabeled trigger sequence \textit{in vitro}
transcription templates were prepared by overlap extension PCR using the primers listed in
Table S2 and purified using Nanosep centrifugal filter columns with a 10 kDa MWCO. 5’-biotinylated
trigger sequence \textit{in vitro} transcription templates were generated by PCR using the unlabeled toehold
trigger \textit{in vitro} transcription template as the template, forward primer 5’-biotin-CGA GGC AGA TCT
TAA TAC GAC TCA CTA TAG-3’ and the appropriate reverse primer from Table S2. All \textit{in vitro}
transcription templates were purified using Nanosep centrifugal filter columns with a 10 kDa
MWCO.

**Preparation of DNA template arrays**

Between 1.75 nM and 224 nM (see Figure legends for details) of 5’ biotinylated, DNA \textit{in vitro}
transcription template in PBS was spotted onto a streptavidin-coated DNA capture surface using an
automated arrayer (Genetix Qarray 2). 4 x 4 fields were spotted using a 175 \( \mu \)m pin head, allowing for a spot separation of 1125 \( \mu \)m. Spotted slides were incubated in a humidity chamber at 37°C for 20 minutes then washed and dried using a standard wash and dry step performed as follows: a wash with 50 ml PBST (PBS containing 0.02% (v/v) Tween 20) at room temperature, with rocking, for 5 minutes followed by a wash with 50 ml MilliQ H\( _2 \)O at room temperature, with rocking, for 30 seconds and a final rinse with MilliQ H\( _2 \)O. The slides were placed in a 50 ml Falcon tube and dried by centrifugation at 180 g, at room temperature for 30 seconds.

**Generation of mRNA arrays**

A DNA template array and streptavidin-coated RNA capture slide were mounted onto the slide separator device described in Figure S6 and assembled in a sandwich format with 12 \( \mu \)l of *in vitro* transcription mixture (3.25 \( \mu \)l of NTP-buffer mix, 0.75 \( \mu \)l of T7 RNA polymerase enzyme and 8 \( \mu \)l nuclease-free H\( _2 \)O; HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB)) as the aqueous filling. For toehold switch experiments requiring trigger RNA free in solution, the *in vitro* transcription mixture was supplemented with 200 nM of unlabeled DNA *in vitro* transcription template encoding the trigger RNA. The sandwich assembly was incubated in a humidity chamber at 37°C for 15 minutes. The slides were separated in MilliQ H\( _2 \)O and the newly generated mRNA array was washed and dried using the standard wash and dry step.

**SpyCatcher protein expression and purification**

The His\(_{6}\)-SpyCatcher expression plasmid, pDEST14-SpyCatcher, was obtained from Prof. Mark Howarth (University of Oxford, UK). *E. coli* BL21(DE3) transformed with pDEST14-SpyCatcher was grown in 0.5 L LB supplemented with 100 \( \mu \)g/ml ampicillin at 37°C, with shaking, until the culture reached an OD\(_{600}\) of 0.5. Isopropyl \( \beta \)-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM and the culture was incubated at 37°C, with shaking, for a further 3 hours. Cells were harvested by centrifugation at 12,000 g, at 4°C for 30 minutes and resuspended in 50ml of
lysis/binding buffer (20 mM Na₂HPO₄ pH 7.4, 0.5 M NaCl, 50 mM imidazole) supplemented with 1 mg/ml lysozyme, 1 U/ml DNase I and one Complete protease inhibitor tablet (Roche). Cells were lysed by brief homogenization followed by sonication. The lysate was clarified by centrifugation at 40,000 g at 4°C for 30 minutes and loaded onto a HisTrap FF column (GE Healthcare) equilibrated in lysis/binding buffer using an ÄKTA Purifier. His₆-SpyCatcher was eluted using a 10 column-volume gradient to 100% elution buffer (20 mM Na₂HPO₄ pH 7.4, 0.5 M NaCl, 500 mM imidazole). The N-terminal hexahistidine tag was removed by TEV cleavage (1 U TEV per 1 µg His₆-SpyCatcher) at room temperature for 15 hours using the ProTEV Plus Kit (Promega). SpyCatcher was concentrated to a final volume of 0.5 ml using a Vivaspin centrifugal concentrator with a MWCO of 10 kDa (Sartorius). Concentrated SpyCatcher was loaded onto a HiLoad 16/600 Superdex 75 prep grade size exclusion column (GE Healthcare) equilibrated in 1X PBS using an ÄKTA Purifier. SpyCatcher was eluted in 1X PBS and aliquots were stored at -70°C.

**mCherry-SpyTag protein expression and purification**

mCherry-SpyTag was expressed from pET28-mCherry-SpyTag and purified essentially as described for SpyCatcher.
Supporting References

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Table S1. DNA sequences of plasmids generated during this study. Sequence elements are colored as follows: T7 promoter – green; 5' UTRs/toehold switches – grey; hexahistidine tag – pink; SpyTag peptide – purple; linker sequence – cyan; streptavidin aptamer – orange; mCherry – red. Restriction sites are italicized and underlined.

| Construct | DNA sequence |
|-----------|--------------|
| pET28-SpyTag<sub>sa</sub> | ...AGATCTTAATACGACTCACTATAAGGGCTTTAAGTTAAGGGGAGAAAAATATGACAACACCACCCACACCCACGCGGGGATGCTAGCATACCCCTCTCTCTAAACCGAGGGGCTTTTCCTCGAG... |
| pET28-mCherry-SpyTag<sub>sa</sub> | ...AGATCTTAATAGACTCACTATAAGGGCTTTAAGTTAAGGGGAGAAAAATATGACAACACCACCCACACCCACGCGGGGATGCTAGCATACCCCTCTCTCTAAACCGAGGGGCTTTTCCTCGAG... |
| pET28-toehold X-mCherry-SpyTag<sub>sa</sub> | ...AGATCTTAATACGACTCACTATAN<sub>N</sub>AGGGCTTTAAGTTAAGGGGAGAAAAATATGACAACACCACCCACACCCACGCGGGGATGCTAGCATACCCCTCTCTCTAAACCGAGGGGCTTTTCCTCGAG... |

\[ X = 1: (N)_{1} = \text{GGGCTTTATACCTATCTCTGTTTTACCTCCTCGATAACAAACAGAGAGATATGCAATGATAAACGAGAACCTGGCGGCAGCGCAAAAG} \]

\[ X = 3: (N)_{3} = \text{GGGATCTATTACTTACCTACATTGCTTTACTCCTCGATAACAAACAGAGAGATATGCAATGATAAACGAGAACCTGGCGGCAGCGCAAAAGATGCGTAAGC} \]

\[ X = 8: (N)_{8} = \text{GGGACCTCTACTTACCTACATTGCTTTACTCCTCGATAACAAACAGAGAGATATGCAATGATAAACGAGAACCTGGCGGCAGCGCAAAAG} \]

\[ X = 9: (N)_{9} = \text{GGGCTTTATACCTATCTCTGTTTTACCTCCTCGATAACAAACAGAGAGATATGCAATGATAAACGAGAACCTGGCGGCAGCGCAAAAG} \]
**Table S2. Primers for overlap extension PCR.** Sequences corresponding to the toehold switches are shown in grey and flanking sequences, with homology to pET28-mCherry-SpyTag\textsubscript{sa}, are shown in blue. Sequences corresponding to the triggers are shown in red.

| Construct | Primer Sequences |
|-----------|------------------|
| Toehold switch 1 PCR product | Forward 1: 5’-GAT GCG TCC GGC GTA GAG GAT CAA ACT TAC CAC TAT AG-3’
| | Forward 2: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
| Toehold switch 3 PCR product | Forward 1: 5’-GAT GCG TCC GGC GTA GAG GAT CAA ACT TAC CAC TAT AG-3’
| | Forward 2: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
| Toehold switch 8 PCR product | Forward 1: 5’-GAT GCG TCC GGC GTA GAG GAT CAA ACT TAC CAC TAT AG-3’
| | Forward 2: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
| Toehold switch 9 PCR product | Forward 1: 5’-GAT GCG TCC GGC GTA GAG GAT CAA ACT TAC CAC TAT AG-3’
| | Forward 2: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
| Trigger 1 | Forward: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
| Trigger 3 | Forward: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
| Trigger 8 | Forward: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
| Trigger 9 | Forward: 5’-TAA TAC GAC TCA GTA TAG GTT GTT ACT ATG TGT ATG GGG TGG CAC TAT AG-3’
| | Reverse: 5’-CTT ATT CAT TAT CAC TCT ATG TGT ATG GGG CAC TAT AG-3’ |
Table S3. DNA sequences of in vitro transcription templates used during this study. Sequence elements are colored as follows: T7 promoter – green; 5' UTRs/toehold switches – grey; hexahistidine tag – pink; mCherry – red; SpyTag peptide – purple; linker sequence – cyan; streptavidin aptamer – orange; trigger – blue. Restriction sites are italicized and underlined.

| Construct | DNA sequence |
|-----------|--------------|
| mCherry-SpyTag<sub>sa</sub> | CGAGGCAAGACTTTAATACGACTCTACTATAGGGCTTAAGTATAAGGAGAGAGAGAGAGATGGTAACTGGACACACACACACCAACCATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCCTGGGACATCCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACCTGAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCATACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCACACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGCTAGCGCCCACATCGTGATGGTGGAACGCCCTACAAGCCGACGAAGGACCCCGACGACTAGTAAAAGCTTACACACACACACACACACACGCATGCATAACCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGCCGGGATGCATGC |
| toehold 1-mCherry-SpyTag<sub>sa</sub> | CGAGGCAAGACTTTAATACGACTCTACTATAGGGCTTAAGTATAAGGAGAGAGAGATGGTAACTGGACACACACACACCAACCATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCCTGGGACATCCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACCTGAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCATACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCACACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGCTAGCGCCCACATCGTGATGGTGGAACGCCCTACAAGCCGACGAAGGACCCCGACGACTAGTAAAAGCTTACACACACACACACACACACGCATGCATAACCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGCCGGGATGCATGC |
| toehold 3-mCherry-SpyTag<sub>sa</sub> | CGAGGCAAGACTTTAATACGACTCTACTATAGGGCTTAAGTATAAGGAGAGAGAGATGGTAACTGGACACACACACACCAACCATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCCTGGGACATCCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACCTGAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCATACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCACACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGCTAGCGCCCACATCGTGATGGTGGAACGCCCTACAAGCCGACGAAGGACCCCGACGACTAGTAAAAGCTTACACACACACACACACACACGCATGCATAACCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGCCGGGATGCATGC |
| toehold 8-mCherry-SpyTag<sub>sa</sub> | CGAGGCAAGACTTTAATACGACTCTACTATAGGGCTTAAGTATAAGGAGAGAGAGATGGTAACTGGACACACACACACCAACCATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCCTGGGACATCCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACCTGAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCATACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCACACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGCTAGCGCCCACATCGTGATGGTGGAACGCCCTACAAGCCGACGAAGGACCCCGACGACTAGTAAAAGCTTACACACACACACACACACACGCATGCATAACCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGCCGGGATGCATGC |
TCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTA
CCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTAC
GACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCA
ACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAG
GGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAG
GCTAGC
GCCCACATCGTGATGGTGGACGCC
TACAAGCCGACGAAG
TAGTAAAAGCTT
ACACACACACACACACACACACACACACACACACACACACACAATGCCACAGAAGGAGATATCGTATGAGAATCAGGAACCTGGCGGCAGCGCAAAAG
ATGCACCACCACTACACCCCGGACATTCCGATGTCCCATCAATAAGAGCAAGACAATGGTAAG
TAGTAATAGATAAG
Biotin-Trigger 8
CGAGGCGAGATCT
AATACGACTCATAAGGGGACUUCUUUUGCAUAAUGCAUAAAGCACGAGGAUAAACGCAGGAGAUAUACAGGAGAUA
UAGAUAAAGGAUGA
Biotin-Trigger 9
CGAGGCGAGATCT
AATACGACTCATAAGGGGACUUCUUUUGCAUAAUGCAUAAAGCACGAGGAUAAACGCAGGAGAUAUACAGGAGAUA
UAGAUAAAGGAUGA
Biotin-Trigger 9
CGAGGCGAGATCT
AATACGACTCATAAGGGGACUUCUUUUGCAUAAUGCAUAAAGCACGAGGAUAAACGCAGGAGAUAUACAGGAGAUA
UAGAUAAAGGAUGA
Figure S1. The SpyCatcher/SpyTag protein coupling system\textsuperscript{19}. (A) A schematic of the SpyCatcher/SpyTag protein coupling system based on PDB 2X5P\textsuperscript{32}. The fibronectin-binding protein FabB from \textit{Streptococcus pyogenes} contains a domain that forms a spontaneous isopeptide bond between a lysine and asparagine. This domain has been split into peptide (SpyTag) and partner protein (SpyCatcher) fragments that rapidly form an amide bond when combined. SpyTag can be fused to proteins of interest, either at the termini or internally, to facilitate their capture via SpyCatcher. (B) Covalent interaction of recombinant mCherry-SpyTag and SpyCatcher. 5 µl reactions containing 1 µg of SpyCatcher and/or 1 µg mCherry-SpyTag in PBS buffer, as indicated, were incubated at room temperature for 30 minutes. Reactions were analyzed by 12% SDS-PAGE and 12% native-PAGE. Mobility shifts under both denaturing and native conditions indicate the formation of a covalent interaction between mCherry-SpyTag and SpyCatcher.
Figure S2. Gene expression analysis of mCherry-SpyTag$_{sa}$. A plot of protein spot intensity against DNA spot intensity for the mCherry-SpyTag$_{sa}$ DNA template and mCherry-SpyTag reporter-protein arrays described in Figure 2. Data were fit to a linear equation.
Figure S3. Schematic of the mechanism of post-transcriptional gene expression regulation by toehold switches. Toehold switches consist of a toehold sequence (red) upstream of a sequence that is capable of forming a hairpin structure and contains the ribosome binding site (RBS; blue), start codon (ATG; purple) and a linker sequence (lilac). They are inserted in-frame, upstream of the coding sequence for the protein of interest (mCherry-SpyTag; green). In the translation “off” state, a hairpin forms that sequesters the ribosome binding site and start codon. In the translation “on” state, a trans-acting, cognate, trigger RNA (cyan) binds the toehold sequence, completes branch migration with the hairpin and releases the ribosome binding site and start codon.
Figure S4. Linear correlation between DNA intensity and spotted DNA concentration for toehold switch-mCherry-SpyTag<sub>sa</sub> templates. Plots of DNA intensity against spotted DNA concentration for the DNA template arrays generated and used in the experiment described in Figure 3.
Figure S5. Linear correlation between RNA intensity and DNA intensity for toehold switch- 
mCherry-SpyTag<sub>sa</sub> mRNAs. Plots of RNA intensity against DNA intensity for the mRNA arrays 
generated and used in the experiment described in Figure 3.
Figure S6. Mounting of the *in vitro* transcription/translation slide sandwiches onto the slide separator assembly. (A) A weight (constructed of glass slides glued together) is attached to the reverse surface of the capture slide and a flat steel bar to the reverse surface of the template slide using removable, heavy duty, double-sided tape (e.g. Gorilla tape). The transcription/translation slide sandwich is assembled and incubated as described in the Methods.

(B) Following *in vitro* transcription/translation, the slide separator assembly is lowered into a reservoir of MilliQ H$_2$O in such a way that the flat steel bar prevents the entire assembly from sinking. Water ingress, promoted by the gravitational pull of the weight, decreases the surface tension inside the slide sandwich, separating the template and capture slides.