**Synthetic circuits that process multiple light and chemical signal inputs**

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**Construction of plasmids**

We list the plasmids used in this study in Table S1

To construct mCherry-TCP fusion and mCherry-NLS-TCP fusion expressing vectors, we used PCR to insert TCP or NLS-TCP coding sequence into mCherry ORF. Specifically, we used forward primer XbaI-mCherry-F and reverse primer EcoRI-TCP1-mcherry-R to amplify mCherry coding sequence from the plasmid BBa\_1712028 (iGEM collections). We obtained mCherry-TCP fusion fragment. On the other hand, we used forward primer XbaI-mCherry-F and reverse primer EcoRI-TCP1-mcherryNLS-R to amplify mCherry-NLS from the plasmid BBa\_1712028, and obtained mCherry-NLS-TCP fusion fragment. We digested the two fragments with XbaI and EcoRI, and then inserted them into PUC19 vector, respectively, for sequencing. Next, we used XbaI and EcoRI to remove mCherry-TCP and mCherry-NLS-TCP from the relevant PUC19 derived vectors. Next, we inserted these two fragments into pcDNA3.1 vector through NheI and EcoRI sites.

To construct a rtTAm constitutive expression vector, we generated a reverse TetR variant that can perform conformational change upon TCP binding to its inducer-binding pockets. The new conformation of reverse TetR allows it to bind to its cognate DNA sequence. Specifically, we introduced five mutations, i.e., E15A-L17G-L25V-M59I-S92R-H93Y to the wild type TetR *(*[*1*](#_ENREF_1)*)*. We used Gibson Assembly Cloning Kit (NEB, catalog number E5510S) to assemble the four PCR fragments containing the desired mutations to obtain the reverse TetR variant. A TetR coding plasmid pcDNA6/TR (Life Technologies) was used as PCR temple. The relevant primers are listed in Table S2. Sequencing confirmed the mutations. Next, we inserted 3 × VP16 coding sequence into the ORF of this reverse TetR at the C-terminal NdeI site. We called this reverse TetR variant and 3×VP16 fusion as rtTAm, which was placed downstream of a CMV promoter.

We constructed two versions of rtTAm-responsible expression vectors, one is TRE3G promoter driving expression of hrGFP, the other is TRE3G promoter driving expression of luciferase. We cloned TRE3G promoter (Clontech) into pU5-hrGFP ([2](#_ENREF_2)) at KpnI and HindIII sites to replace the U5 promoter. We also inserted the TRE3G promoter into pGL3-Basic (Promega) at SmaI and NcoI sites. Then we cut out the TRE3G promoter-luciferase cassette by digesting the plasmid with NheI and SalI and inserted this cassette into pBX-023 ([3](#_ENREF_3)), obtained pBX-TRE3G-luciferase.

We combined light-inducible mCherry-TCP expression cassette and cumate-inducible rtTAm expression cassette into a single vector. We first cloned rtTAm from the pCMV-rtTAm vector and inserted it into QM521A (System Biosciences, Cumate-switch system) at NheI and NotI sites. Then, we replaced the coGFP gene in QM521A with an EYFP gene. After this modification, rtTAm and EYFP were integrated into one ORF linked by 2A peptide. Next, we digested the pCMVCuO5-rtTAm-2A-EYFP plasmid with sfiI and HpaI and treated the fragment with T4 DNA Polymerase (NEB, catalog number M0203S) to generate blunt ends. The prepared fragment was then inserted into pU5-mCherry-TCP at MfeI site. We digested pU5-mCherry-TCP with MfeI and treated the linearized plasmid with T4 DNA Polymerase and Alkaline Phosphatase, Calf Intestinal (NEB, catalog number M0290S) before ligated it with the pCMVCuO5-rtTAm-2A-EYFP fragment. Since it was a blunt ends ligation, these two cassettes could be either be “face-to-face” or “back-to-back” ligated. We chose a “back-to-back” connected version for the followed experiments.

We established a conditional positive feedback plasmid. We first generated a TetR and 3 × VP16 fusion by fused 3 × VP16 sequence at the C-terminal end of TetR (TAA was removed) by PCR. We called this fusion as tTA. Then we placed the tTA gene downstream of TRE3G promoter via BamHI and MfeI digestion.

**Table S1.** Key plasmids used in this study

|  |  |  |
| --- | --- | --- |
| Vector | Description | Reference  |
| QM200PApBX-GAVPO-zeocinpCMV-mCherry-TCPpCMV-mCherry-NLS-TCPpCMV-rtTAmpTRE3G-hrGFPpU5-mCherry-TCP pBX-TRE3G-luciferasepU5-mCherry-TCP-CMVCuO5-rtTAm-2A-EYFPpTRE3G-tTA | CymR and Puromycin resistance gene are co-expressed by EF1a promoterThe coding sequence of GAVPO and Zeocin linked with 2A peptide. This cassette is flanked by 5’ and 3’terminal repeats of PiggyBac transposon and HS4 insulator sequence.CMV promoter drives expression of mCherry-TCP fusionCMV promoter drives expression of mCherry-NLS-TCP fusionCMV promoter drives expression of reverse TetR variant and 3×VP16 fusionTRE3G promoter drives expression of the humanized recombinant GFP (hrGFP)GAVPO-responsible promoter U5 drives expression of mCherry-TCP fusionTRE3G-promoter drives expression of luciferase. This cassette is flanked by 5’ and 3’terminal repeats of PiggyBac transposon and HS4 insulator sequence.GAVPO-responsible promoter U5 drives expression of mCherry-TCP fusion, and CMVCuO5 promoter drives co-expression of rtTAm and EYFP. These two cassettes are combined into one plasmid. A hygromycin resistant gene driven by SV40 promoter is also encoded in this plasmid. TRE3G promoter derives expression of TetR and 3×VP16 fusion | System Biosciences (SBI)Unpublished dataThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis study |

**Table S2.** Key oligonucleotides used in this study

|  |  |  |
| --- | --- | --- |
| Name | Sequence | Use |
| XbaI-mcherry-FEcoRI-TCP1-mcherry-REcoRI-TCP1-mcherryNLS-RRevTetR-SLIC-1FRevTetR-SLIC-1RRevTetR-SLIC-2FRevTetR-SLIC-2RRevTetR-SLIC-3FRevTetR-SLIC-3RRevTetR-SLIC-4FRevTetR-SLIC-4R | TATGTCTAGAGCCACCATGGTGAGCAAGGGCGAGGAGTGAATTCTTAGTTCCAGCTGGGCAGCAGGCGGGCCACGGCCATGATGATCTTGCCGGTCTTGTACAGCTCGTCCGTGAATTCAGTTCCAGCTGGGCAGCAGGCGGGCCACGGCCATGATGATCTTGCCGGTTACCTTTCTCTTCTTTTTCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGTACCCTCACACCTTCGATTCCGACCTCATTGCCCAGGGCTAATGCCCCTGGGCAATGAGGTCGGAATCGAAGGTGTGACAACCCTAAGATCTCAATGGCTAAGGCGTCGAGCATGCTCGACGCCTTAGCCATTGAGATCTTAGATAGGCCTTTTGCTCCATCGCGATATCTTAGTAAAGCGCTTTACTAAGATATCGCGATGGAGCAAAAGAAACAAGTTCTGCTTTAATAAGATCTGAATTCC | mCherry cloningAdd TCP to mCherryAdd TCP to mCherry-NLSIntroduce E15A-L17G-L25V-M59I-S92R-H93Y to TetR |

**Sequence information**

**CMV(tetO2) promoter**

TATA box 573-579 bp

2 × tetO 589-628 bp

 1 GTTGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC TTACGGTAAA

 CAACTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG AATGCCATTT

 101 TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA

 ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT AACTGCAGTT

 201 TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT AAATGGCCCG

 ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA TTTACCGGGC

 301 CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA

 GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG CCAAAACCGT

 401 GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGAACCA AAATCAACGG

 CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCTTGGT TTTAGTTGCC

 501 GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCTC CCTATCAGTG

 CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGAG GGATAGTCAC

 601 ATAGAGATCT CCCTATCAGT GATAGAGATC GTCGACGAGC TCGTTTAGTG AACCGTCAGA TCGCCTGGAG ACGCCATCCA CGCTGTTTTG ACCTCCATAG

 TATCTCTAGA GGGATAGTCA CTATCTCTAG CAGCTGCTCG AGCAAATCAC TTGGCAGTCT AGCGGACCTC TGCGGTAGGT GCGACAAAAC TGGAGGTATC

 701 AAGACACCGG GACCGATCCA GCCTCCG

 TTCTGTGGCC CTGGCTAGGT CGGAGGC

**CMV5(CuO) promoter**

CuO element 594-620 bp

 1 AGACTAGTTA TTAATAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC ATAACTTACG GTAAATGGCC CGCCTGGCTG

 TCTGATCAAT AATTATCATT AGTTAATGCC CCAGTAATCA AGTATCGGGT ATATACCTCA AGGCGCAATG TATTGAATGC CATTTACCGG GCGGACCGAC

 101 ACCGCCCAAC GACCCCCGCC CATTGACGTC AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT GGAGTATTTA

 TGGCGGGTTG CTGGGGGCGG GTAACTGCAG TTATTACTGC ATACAAGGGT ATCATTGCGG TTATCCCTGA AAGGTAACTG CAGTTACCCA CCTCATAAAT

 201 CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTCC GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC

 GCCATTTGAC GGGTGAACCG TCATGTAGTT CACATAGTAT ACGGTTCAGG CGGGGGATAA CTGCAGTTAC TGCCATTTAC CGGGCGGACC GTAATACGGG

 301 AGTACATGAC CTTACGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT GATGCGGTTT TGGCAGTACA CCAATGGGCG

 TCATGTACTG GAATGCCCTG AAAGGATGAA CCGTCATGTA GATGCATAAT CAGTAGCGAT AATGGTACCA CTACGCCAAA ACCGTCATGT GGTTACCCGC

 401 TGGATAGCGG TTTGACTCAC GGGGATTTCC AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG CACCAAAATC AACGGGACTT TCCAAAATGT

 ACCTATCGCC AAACTGAGTG CCCCTAAAGG TTCAGAGGTG GGGTAACTGC AGTTACCCTC AAACAAAACC GTGGTTTTAG TTGCCCTGAA AGGTTTTACA

 501 CGTAATAACC CCGCCCCGTT GACGCAAATG GGCAAGCTTG CCGGGTCGAG GTAGGCGTGT ACGGTGGGAG GCCTATATAA GCAACCGGTA TAATACAAAC

 GCATTATTGG GGCGGGGCAA CTGCGTTTAC CCGTTCGAAC GGCCCAGCTC CATCCGCACA TGCCACCCTC CGGATATATT CGTTGGCCAT ATTATGTTTG

 601 AGACCAGATT GTCTGTTTGT TACCGGTGTT TAGTGAACCG GGCGCGCCTC ATATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA TAGAAGACAC

 TCTGGTCTAA CAGACAAACA ATGGCCACAA ATCACTTGGC CCGCGCGGAG TATAGCGGAC CTCTGCGGTA GGTGCGACAA AACTGGAGGT ATCTTCTGTG

 701 CGGGACCGAT CCAGCCTCCG CGGTCACTCT CTTCCGCATC GCTGTCTGCG AGGGCCAGCT GTTGGGCTCG CGGTTGAGGA CAAACTCTTC GCGGTCTTTC

 GCCCTGGCTA GGTCGGAGGC GCCAGTGAGA GAAGGCGTAG CGACAGACGC TCCCGGTCGA CAACCCGAGC GCCAACTCCT GTTTGAGAAG CGCCAGAAAG

 801 CAGTACTCTT GGATCGGAAA CCCGTCGGCC TCCGAACGGT ACTCCGCCAC CGAGGGACCT GAGCCAGTCC GCATCGACCG GATCGGAAAA CCTCTCGAGA

 GTCATGAGAA CCTAGCCTTT GGGCAGCCGG AGGCTTGCCA TGAGGCGGTG GCTCCCTGGA CTCGGTCAGG CGTAGCTGGC CTAGCCTTTT GGAGAGCTCT

 901 AAGGCGTCTA ACCAGTCACA GTCGCAAGGT AGGCTGAGCA CCGTGGCGGG CGGCAGCGGG TGGCGGTCGG GGTTGTTTCT GGCGGAGGTG CTGCTGATGA

 TTCCGCAGAT TGGTCAGTGT CAGCGTTCCA TCCGACTCGT GGCACCGCCC GCCGTCGCCC ACCGCCAGCC CCAACAAAGA CCGCCTCCAC GACGACTACT

 1001 TGTAATTAAA GTAGGCGGTC TTGAGCCGGC GGATGGTCGA GGTGAGGTGT GGCAGGCTTG AGATCCAGCT GTTGGGGTGA GTACTCCCTC TCAAAAGCGG

 ACATTAATTT CATCCGCCAG AACTCGGCCG CCTACCAGCT CCACTCCACA CCGTCCGAAC TCTAGGTCGA CAACCCCACT CATGAGGGAG AGTTTTCGCC

 1101 GCATGACTTC TGCGCTAAGA TTGTCAGTTT CCAAAAACGA GGAGGATTTG ATATTCACCT GGCCCGATCT GGCCATACAC TTGAGTGACA ATGACATCCA

 CGTACTGAAG ACGCGATTCT AACAGTCAAA GGTTTTTGCT CCTCCTAAAC TATAAGTGGA CCGGGCTAGA CCGGTATGTG AACTCACTGT TACTGTAGGT

 1201 CTTTGCCTTT CTCTCCACAG GTGTCCACTC CCAGGTCCAA GTTT

 GAAACGGAAA GAGAGGTGTC CACAGGTGAG GGTCCAGGTT CAAA

**Amino acid sequence of rtTAm**

Reverse tetR variant 1-206 aa

3 × VP16 207-249 aa

MSRLDKSKVINSALALGNEVGIEGVTTRKLAQKLGVEQPTLYWHVKNKRALLDALAIEILDRHHTHFCPLEGESWQDFLRNNAKSFRCALLRYRDGAKVHLGTRPTEKQYETLENQLAFLCQQGFSLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDSMPPLLRQAIELFDHQGAEPAFLFGLELIICGLEKQLKCESGGPTDALDDFDLDMLPADALDDFDLDMLPADALDDFDLDMLPG

**Amino acid sequence of TCP**

TGKIIMAVARLLPSWN

**Amino acid sequence of mCherry-TCP**

MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKTGKIIMAVARLLPSWN

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