Electronic Supplementary Material (ESI) for Molecular BioSystems.

Electronic Supplementary Information

For

Ligand-responsive upregulation of 3' CITE-mediated translation in a wheat germ cell-free expression system

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Preparation of DNA Templates. DNA templates for *in vitro* transcription were prepared with sequential polymerase chain reactions (PCRs) by using PrimeSTAR Max DNA Polymerase, which has extremely high fidelity, from Takara Bio (Ohtsu, Japan). Most of the PCR primers were purchased from Thermo Fisher Scientific (Tokyo, Japan). PCR primers for the final PCR were synthesized and purified with an oligonucleotide purification cartridge by Eurofins Genomics (Tokyo, Japan). The primer sequences are described below. A plasmid encoding the Ypet gene (pHis-SRY-YPet)[1] or a plasmid encoding the NanoLuc (nLuc) gene (pEU-nLuc-Venus), the latter of which was kindly donated by Dr. Hiroyuki Takeda of Ehime University, was used as a PCR template in all 1st PCRs. All PCR products except those in the final PCR were agarose gel-purified and then used as a template in the next PCR.

Sequences of PCR Primers.

(written from 5' (left) to 3' (right); shaded: non-original bases; red: bases for implanting aptamers; purple: bases for introducing mutations into aptamers).

Forward primer in the 1st PCR for **BY**, **5SL-BY**, **BYm** and **5SL-BYm**

CCCTTATTGCCTGACAAGCTAAGGGCCACCCTTCTTTCCCCACCGCCATCATGGCCCATCAC
CATCACCATCAT

Forward primer in the 1st PCR for **BYm2**, **5SL-BYm2**, **5SL-BYm2-theo** and **5SL-BYm2-theo^{mut}**

CCCTTATTGCCTGGCAAGCTAAGGGCCACCCTTCTTTCCCCACCGCCATCATGGCCCATCAC
CATCACCATCAT

Reverse primer in the 1st PCR for **BY**, **5SL-BY**, **BYm**, **5SL-BYm**, **BYm2** and **5SL-BYm2**

GCCTGTTTCCCAGGATCCGATTTGTGCTAGTGGTGTTGTCTTTACTTGTACAGCTCGTTCAT
GCCCTCA

Reverse primer in the 1st PCR for **5SL-BYm2-theo** and **5SL-BYm2-theo^{mut}**

GCCTGTTTCCCAGGATCCGATTTGTTGGTATCCCTGTTGTCTTTACTTGTACAGCTCGTTCAT
GCCCTCA

Forward primer in the 2nd PCR for **BY**, **5SL-BY**, **BYm** and **5SL-BYm**

GTGCTTGTAACACACTACGCGCCCGTTTTGTATTCGGGAAGTAGTTGCGAAAACGGTCCCC
TTATTGCCTGACAAGCTA

Forward primer in the 2nd PCR for **BYm2**, **5SL-BYm2**, **5SL-BYm2-theo** and **5SL-BYm2-theo^{mut}**

GTGCTTGTAACACACTACGCGCCCGTTTTGTATTCGGGAAGTAGTTGCGAAAACGGTCCCC
TTATTGCCTGGCAAGCTA

Reverse primer in the 2nd PCR for **BY** and **5SL-BY**

TACGGCGGTAGGTTGACGGCCTACCCGAGCTTACGAACCGAAGTTCTGCCTGTTTCCCAGG
ATCCGATTTG

Reverse primer in the 2nd PCR for **BYm** and **5SL-BYm**

TACGGCGGTAGGTTGACAGCCTACCCGAGCTTATGAACCGAAGTTCTGCCTGTTTCCCAGG
ATCCGATTTG

Reverse primer in the 2nd PCR for **BYm2**, **5SL-BYm2**, **5SL-BYm2-theo** and **5SL-BYm2-theo^{mut}**

TACGATACGGCGGTAGGTTGGCAGCCTACCCGAGCTTATGAACCGAAGTTCTGCCTGTTTC
CCAGGATCCGATTTG

Forward primer in the 3rd (final for **BY**, **BYm** and **BYm2**) PCR for **BY**, **5SL-BY**, **BYm**, **5SL-BYm**, **BYm2**, **5SL-BYm2**, **5SL-BYm2-theo** and **5SL-BYm2-theo^{mut}**

CATACGATTTAGGTGACACTATAGAAGTGAAGATTGACCATCTCACAAAAGCTGTTACGTG
CTTGTAACACACTACG

Reverse primer in the 3rd (final for **BY** and **BYm**) PCR for **BY**, **5SL-BY**, **BYm**, **5SL-BYm**

GGCCAAACACAATACGATACGGCGGTAGGTTGAC

Reverse primer in the 3rd (final for **BYm2**) PCR for **BYm2** and **5SL-BYm2**

GGCCAAACACAATACGATACGGCGGTAGGTTG

Reverse primer in the 3rd PCR for **5SL-BYm2-theo**

GGGCTGCCAAGGACAATACGATACGGCGGTAGGTTG

Reverse primer in the 3rd PCR for **5SL-BYm2-theo^{mut}**

GGGCTGCCTATGACAATACGATACGGCGGTAGGTTG

Forward primer in the 4th (final) PCR for **5SL-BY**, **5SL-BYm**, **5SL-BYm2**, **5SL-BYm2-theo** and **5SL-BYm2-theo^{mut}**

GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCGAAGTGAAGATTGACCATCTCA

Reverse primer in the 4th (final) PCR for **5SL-BY** and **5SL-BYm**

GGCCAAACACAATACGATACGGCGGTAGGTTGAC

Reverse primer in the 4th (final) PCR for **5SL-BYm2**

GGCCAAACACAATACGATACGGCGGTAGGTTG

Reverse primer in the 4th (final) PCR for **5SL-BYm2-theo**

GGGCTGCCAAGGACAATACGATACGGCGGTAGGTTG

Reverse primer in the 4th (final) PCR for **5SL-BYm2-theo^{mut}**

GGGCTGCCTATGACAATACGATACGGCGGTAGGTTG

Forward primer in the 1st (final) PCR for **pMK-106**

CATACGATTTAGGTGACACTATAG

Reverse primer in the 1st (final) PCR for **pMK-106**

ATACGCAAGGAAACAGCTATGAC

Forward primer in the 1st (final) PCR for **5SL-pMK-106**

GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCGAACTCACCTATCTCCCC

AACAC

Reverse primer in the 1st (final) PCR for **5SL-pMK-106**

ATACGCAAGGAAACAGCTATGAC

Forward primer in the 1st PCR for **5SL-pMK-106(nLuc)**

CATTCAATCACTCTTTCCACTAACCACCTATCTACATCACCAAGATATCACTAGTATGGTCT

TCACACTCGAAGATTTC

Reverse primer in the 1st PCR for **5SL-pMK-106(nLuc)**

TCCCGACTGGAAAGCGGGCAGTGAAAGGAAGGCCCATGAGGCCCAGGAGCTCGAGTTACG

CCAGAATGCGTTCGCACAG

Forward primer in the 2nd PCR for **5SL-pMK-106(nLuc)**

CATACGATTTAGGTGACACTATAGAACTCACCTATCTCCCCAACACCTAATAACATTCAAT

CACTCTTTCCACTAAC

Reverse primer in the 2nd PCR for **5SL-pMK-106(nLuc)**

ATACGCAAGGAAACAGCTATGACCATGTTAATGCAGCTGGCACGACAGGTTTCCCGACTGG

AAAGCGGGCAGTGAA

Forward primer in the 3rd (final) PCR for **5SL-pMK-106(nLuc)**

GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCGAACTCACCTATCTCCCC

AACAC

Reverse primer in the 3rd (final) PCR for **5SL-pMK-106(nLuc)**

ATACGCAAGGAAACAGCTATGAC

Forward primer in the 1st PCR for **5SL-BYm2(nLuc)**, **5SL-BYm2-theo(nLuc)**, **5SL-BYm2-theo^{mut}(nLuc)** and **5SL-BYm2-TMR(nLuc)**

CCCTTATTGCCTGGCAAGCTAAGGGCCACCCTTCTTTCCCCACCGCCATCATGGTCTTCACA

CTCGAAGATTTC

Reverse primer in the 1st PCR for **5SL-BYm2(nLuc)**

GCCTGTTTCCCAGGATCCGATTTGTGCTAGTGGTGTTGTCTTTACGCCAGAATGCGTTCGCA

CAG

Reverse primer in the 1st PCR for **5SL-BYm2-theo(nLuc)** and **5SL-BYm2-theo^{mut}(nLuc)**

GCCTGTTTCCCAGGATCCGATTTGTTGGTATCCCTGTTGTCTTTACGCCAGAATGCGTTCGC

ACAG

Reverse primer in the 1st PCR for **5SL-BYm2-TMR(nLuc)**

GCCTGTTTCCCAGGATCCGATTTGTCACTCTTTACCCTGTTGTCTTTACGCCAGAATGCGTTC

GCACAG

Forward primer in the 2nd PCR for **5SL-BYm2(nLuc)**, **5SL-BYm2-theo(nLuc)**, **5SL-BYm2-theo^{mut}(nLuc)** and **5SL-BYm2-TMR(nLuc)**

GTGCTTGTAACACACTACGCGCCCGTTTTGTATTCGGGAAGTAGTTGCGAAAACGGTCCCC

TTATTGCCTGGCAAGCTA

Reverse primer in the 2nd PCR for **5SL-BYm2(nLuc)**, **5SL-BYm2-theo(nLuc)**, **5SL-BYm2-theo^{mut}(nLuc)** and **5SL-BYm2-TMR(nLuc)**

TACGATACGGCGGTAGGTTGGCAGCCTACCCGAGCTTATGAACCGAAGTTCTGCCTGTTTC

CCAGGATCCGATTTG

Forward primer in the 3rd PCR for **5SL-BYm2(nLuc)**, **5SL-BYm2-theo(nLuc)**, **5SL-BYm2-theo^{mut}(nLuc)** and **5SL-BYm2-TMR(nLuc)**

CATACGATTTAGGTGACACTATAGAAGTGAAGATTGACCATCTCACAAAAGCTGTTACGTG

CTTGTAACACACTACG

Reverse primer in the 3rd PCR for **5SL-BYm2(nLuc)**

GGCCAAACACAATACGATACGGCGGTAGGTT

Reverse primer in the 3rd PCR for **5SL-BYm2-theo(nLuc)**

GGGCTGCCAAGGACAATACGATACGGCGGTAGGTT

Reverse primer in the 3rd PCR for **5SL-BYm2-theo^{mut}(nLuc)**

GGGCTGCCTATGACAATACGATACGGCGGTAGGTT

Reverse primer in the 3rd PCR for **5SL-BYm2-TMR (nLuc)**

GGGAAGACCTCTACAATACGATACGGCGGTAGGTT

Forward primer in the 4th (final) PCR for **5SL-BYm2(nLuc)**, **5SL-BYm2-theo(nLuc)**, **5SL-BYm2-theo^{mut}(nLuc)** and **5SL-BYm2-TMR(nLuc)**

GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCGAAGTGAAGATTGACCAT

CTCA

Reverse primer in the 4th (final) PCR for **5SL-BYm2(nLuc)**

GGCCAAACACAATACGATACGGCGGTAGGTT

Reverse primer in the 4th (final) PCR for **5SL-BYm2-theo(nLuc)**

GGGCTGCCAAGGACAATACGATACGGCGGTAGGTT

Reverse primer in the 4th (final) PCR for **5SL-BYm2-theo^{mut}(nLuc)**

GGGCTGCCTATGACAATACGATACGGCGGTAGGTT

Reverse primer in the 4th (final) PCR for **5SL-BYm2-TMR(nLuc)**

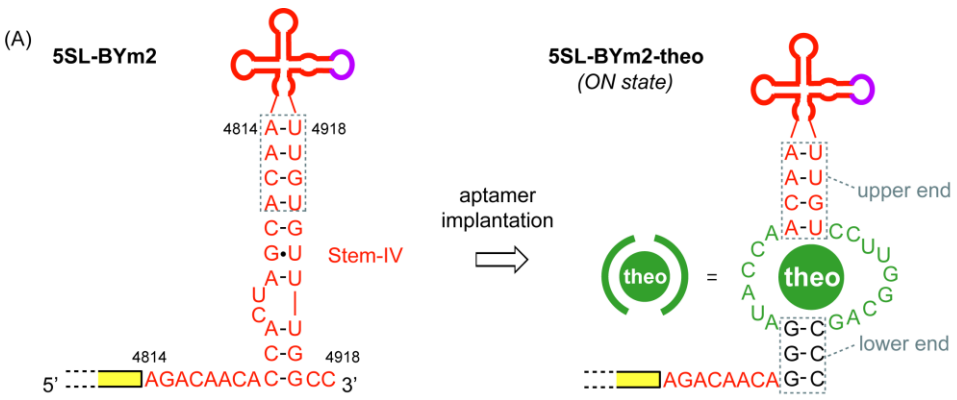
GGGAAGACCTCTACAATACGATACGGCGGTAGGTT

Preparation of mRNA Templates. Run-off transcription of DNA templates was performed with an AmpliScribe SP6 High Yield Transcription Kit (CellScript, Madison, WI) or an AmpliScribe T7 High Yield Transcription Kit (CellScript) for 5'-SL-free mRNAs or 5'-SL-containing mRNAs, respectively, according to the manufacturer's protocol. The transcribed mRNA was purified with an RNeasy MinElute Cleanup Kit (QIAGEN, Tokyo, Japan) and quantified by the absorbance at 260 nm.

Translation of mRNA Templates in WGE. Cell-free translation of mRNA templates was carried out at 26°C for 1 h in batch mode (10 μL) with the WEPRO1240 Expression Kit (CellFree Sciences, Matsuyama, Japan) as previously described.[1,2]

YPet Assay. The fluorescence intensities of translated YPet were measured as previously described.[1]

Luciferase Assay. The translation solution was 1000-fold diluted in two steps with double-distilled water. An aliquot (45 μL) of the diluted solution was mixed with 8 μL of Nano-Glo Luciferase Assay Reagent containing furimazine (Promega, Tokyo, Japan) in a black 96-well plate.[3] The resulting chemiluminescence intensities were measured 3 min after the mixing with a Wallac ARVO MX (Perkin-Elmer, Yokohama, Japan). Chemiluminescence images were acquired using a Light-Capture imaging system (ATTO, Tokyo, Japan).


(A)
5SL-BYm2
4814
4918
Stem-IV
5' AGACAACA C-G CC 3'
aptamer implantation
5SL-BYm2-theo
(ON state)
upper end
lower end
theo
=
AGACAACA


(B)
5SL-BYm2-theo
5SL-BYm2-theo2
5SL-BYm2-theo3
5SL-BYm2-theo4
5SL-BYm2-theo5
5SL-BYm2-theo6
5SL-BYm2-theo7
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theo
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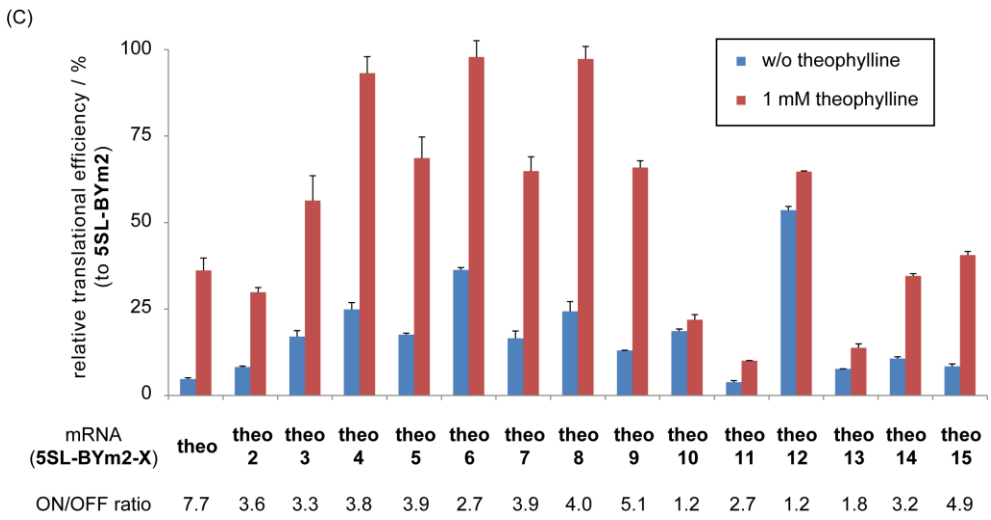

Figure S1. Validation of the design of **5SL-BYm2-theo**. (A) The design strategy for **5SL-BYm2-theo**: a split theophylline aptamer is implanted into Stem-IV of **5SL-BYm2**. Only the ON-state structure is shown for **5SL-BYm2-theo**. (B) Alteration of surrounding bases of the implanted theophylline aptamer in **5SL-BYm2-theo** for the preparation of other 14 riboswitch candidates (**5SL-BYm2-theo2~15**). **5SL-BYm2-theo14** and **5SL-BYm2-theo15** are hybridization switch-based riboswitches, which were constructed by adding a short modulator sequence (MS) to the 3' end by reference to our previous reports.[2,4] (C) The relative translation efficiencies of mRNAs in B in the absence or presence of 1 mM theophylline in WGE.

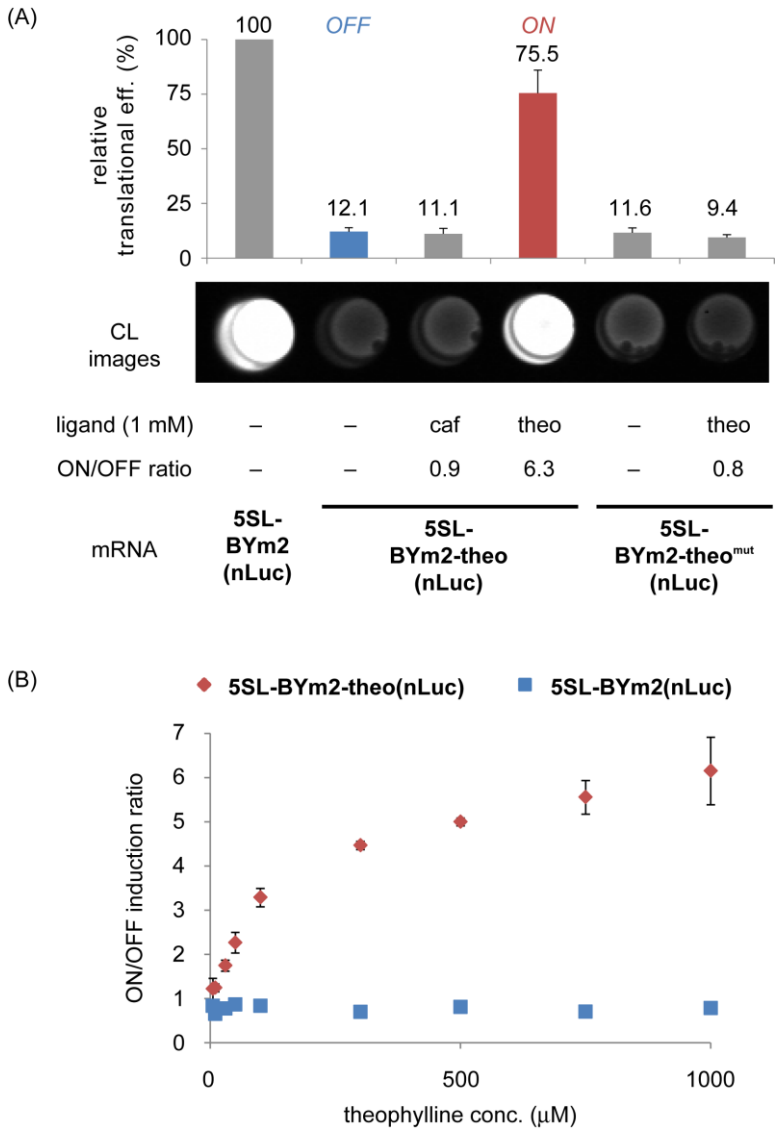

Figure S2. Characterization of **5SL-BYm2-theo(nLuc)**. (A) The relative activities (i.e., translation efficiencies, above) and chemiluminescence images (below) of nLuc translated from **5SL-BYm2-theo(nLuc)** and **5SL-BYm2-theo^{mut}(nLuc)** in the absence or presence of 1 mM theophylline (theo) or caffeine (caf) in WGE. (B) The ON/OFF induction ratios of **5SL-BYm2-theo(nLuc)** and **5SL-BYm2(nLuc)** at various concentrations of theophylline.

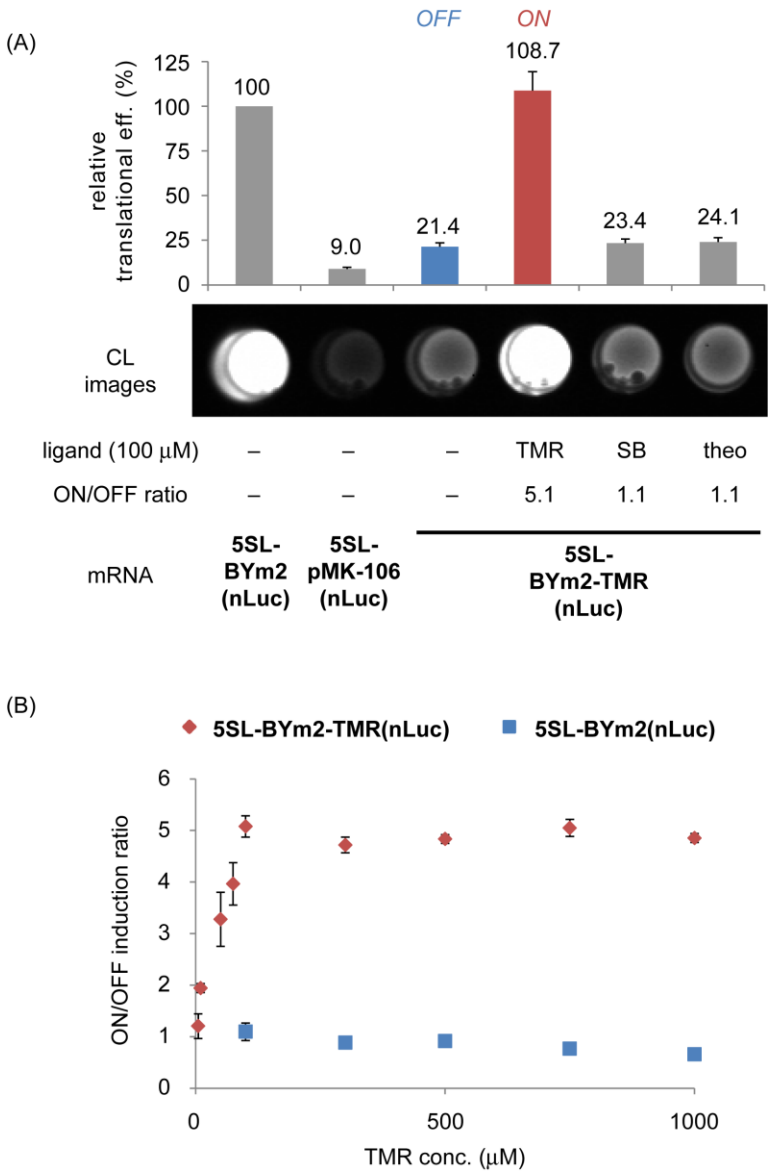

Figure S3. Characterization of **5SL-BYm2-TMR(nLuc)**. (A) The relative activities (i.e., translation efficiencies, above) and chemiluminescence images (below) of nLuc translated from **5SL-BYm2-TMR(nLuc)** in the absence or presence of 100 μM 5-carboxytetramethylrhodamine (TMR), sulforhodamine B (SB) or theophylline (theo) in WGE. (B) The ON/OFF induction ratios of **5SL-BYm2-TMR(nLuc)** and **5SL-BYm2(nLuc)** at various concentrations of TMR.

References.

(1) A. Ogawa, J. Tabuchi and Y. Doi, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 3724.

(2) A. Ogawa, *RNA*, 2011, **17**, 478.

(3) M. P. Hall *et al.*, *ACS Chem. Biol.*, 2012, **7**, 1848.

(4) A. Ogawa and J. Tabuchi, *Org. Biomol. Chem.*, 2015, **13**, 6681.