Electronic Supplementary Material (ESI) for Chemical Communications

Light-activatable molecular beacons with caged loop sequence

Khashti Ballabh Joshi, Andreas Vlachos, Vera Mikat, Thomas Deller and Alexander Heckel

SUPPORTING INFORMATION

1. Oligonucleotide synthesis

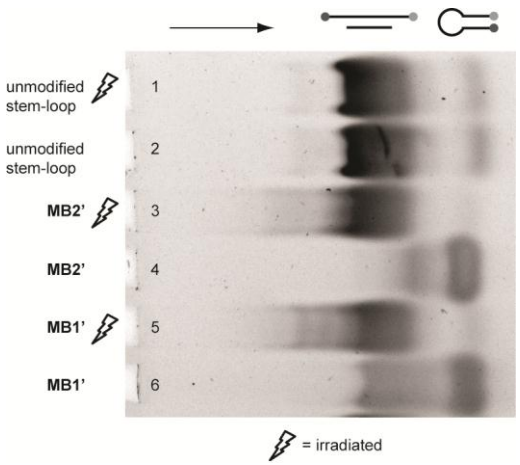
The sequence of the target RNA was 5'-UGG UAU CGU GGA AGG ACU C-3'. Oligonucleotide solid-phase synthesis was performed by IBA GmbH, Göttingen. BHQ-2 was introduced via a modified solid phase. DNA-synthesis was performed DMT-off. After cleavage and deprotection with 33% aq. ammonia (4 h at 65 °C) and evaporation of the solvent the crude product was purified by RP-HPLC. Cy3 was labeled post synthetically to the NH_2-$(CH_2)_6$-modified 5'-end by using GE-Amersham's CyDye labeling kit according to manufacturer's instructions. The labeling product was purified via gel filtration followed by RP-HPLC (conditions for all purifications: 0.1 M triethylammonium acetate, pH 7.4/ acetonitrile gradient 20-35% MeCN, flow: 1 mL/min, column: Agilent's Eclipse XDB-C18, 4.6x250 mm at 55 °C). The purity and identity of the oligonucleotides were confirmed by HPLC-MS (Bruker micrOTOF-Q II).

Sequence	Mass [Da] calcd.	Mass [Da] found
MB1' 5'-<u>CGA CG</u>G AG^{NPP}T CC^{NPE}T TC^{NPE}C ACG^{NPP} ATA C^{NPE}CA <u>CGT CG</u>-3'	**9592.8 (exact)**	**9592.9 (exact)**
MB1 5'-Cy3-NH_2-$(CH_2)_6$-<u>CGA CG</u>G AG^{NPP}T CC^{NPE}T TC^{NPE}C ACG^{NPP} ATA C^{NPE}CA <u>CGT CG</u>-BHQ-2-3'	**10942.9 (avg.)**	**10942.3 (avg.)**
MB2' 5'-<u>CGA CG</u>G AG^{NPP}T CC^{NPE}T TC^{NPE}C A^{NPE}CG^{NPP} ATA^{NPE} CC^{NPE}A <u>CGT CG</u>-3'	**9890.9 (exact)**	**9892.1 (exact)**
MB2 5'-Cy3-NH_2-$(CH_2)_6$-<u>CGA CG</u>G AG^{NPP}T CC^{NPE}T TC^{NPE}C A^{NPE}CG^{NPP} ATA^{NPE} CC^{NPE}A <u>CGT CG</u>-BHQ-2-3'	**11241.2 (avg.)**	**11241.0 (avg.)**

Supplementary Table 1 Synthesized caged sequences. Nucleobases forming the stem are underlined.

2. Polyacrylamide gel electrophoresis (PAGE)

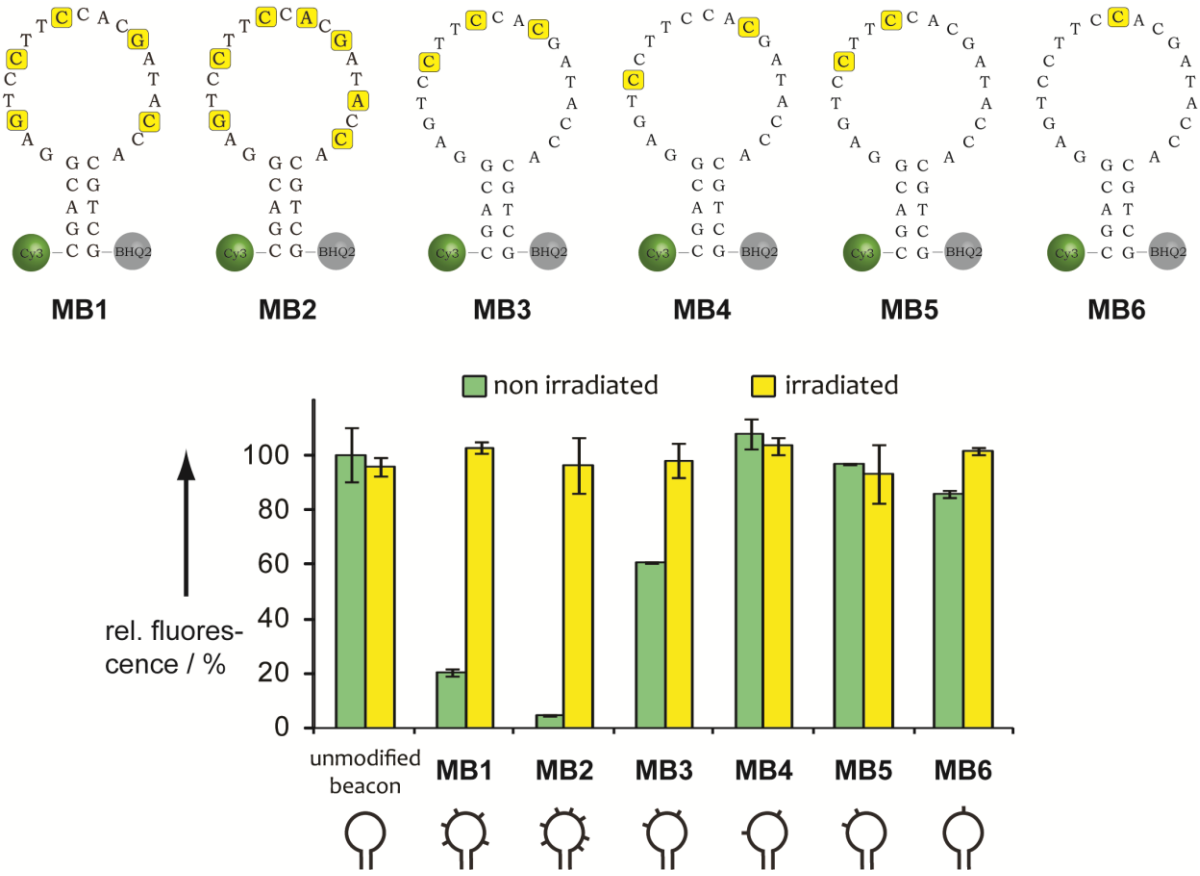
Gel electrophoresis was performed using a non-denaturing 12% polyacrylamide gel in TBE buffer (89 mM Tris/89 mM boric acid/2 mM EDTA, 11 mM $MgCl_2$, pH 8.3). The gel was run at 60 V for 18 hours. To prevent heating of the gel, the run was conducted at 4 °C. Bands were visualized by staining with SYBR Gold (Promega).



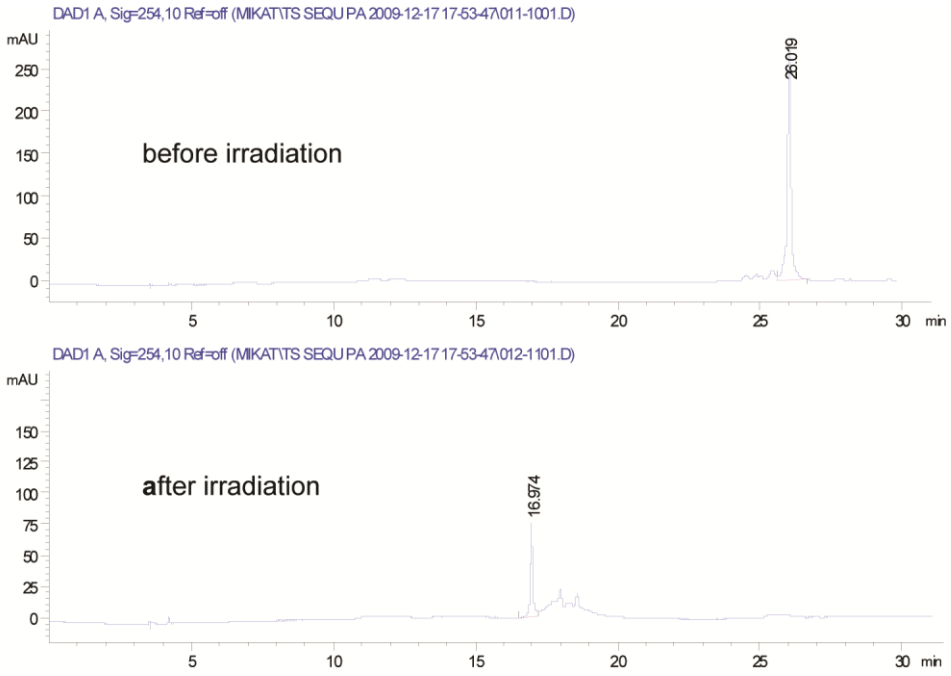
Supplementary Figure 1. Native gel electrophoresis (SYBR Gold) of target RNA mixed with equimolar amounts of stem-loop sequences **MB1'** and **MB2'** in lanes 3-6 and an excess of stem-loop sequences **MB1'** and **MB2'** in lanes 1 and 2. The latter results in a slower band with the hybridized double strand and a faster one with the excess unbound stem-loop oligonucleotide for comparison. Samples in lanes 1, 3 and 5 were irradiated with 366 nm UV-light for 2.5 min prior to loading onto the gel.

3. Fluorescence assay

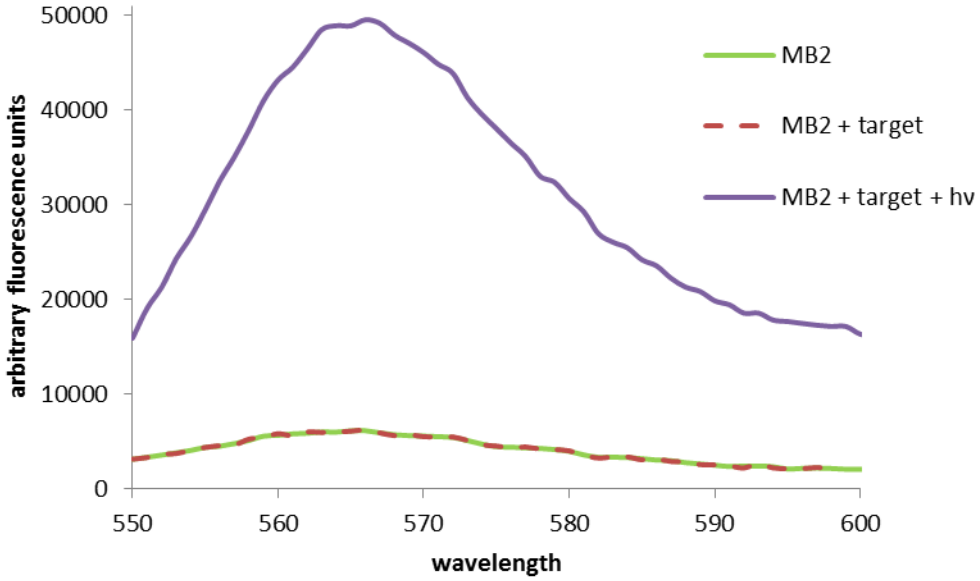
A solution of the molecular beacon (1 µM) was combined with target RNA solution (2 µM) in 1x PBS buffer and incubated for 1 min at 37 °C. Irradiation was performed in 1x PBS buffer using a UV-LED (NICHIA NCCU033(T) for specifications see vendor's data sheet) operated at 0.5 A for 2.5 min. Fluorescence intensity was measured immediately with an infinite 200 plate reader (Tecan).



Supplementary Figure 2. Overview of the results of the fluorescence assays including also further caged molecular beacons with less than five caging groups.



Supplementary Figure 3. HPLC traces (conditions see above) of **MB2'** (seven cages) before and after irradiation under the procedure detailed above.



Supplementary Figure 4. Fluorescence spectra of **MB2** in presence or absence of target sequence with or without prior uncaging. (λ_{ex} = 532 nm; Tecan M-200 pro).

4. Activation of caged beacon with spatial resolution

5'-NH_2-$(CH_2)_6$–modified target RNA was spotted and immobilized on glass-slides with an aldehyde-modified surface using the Nexterion® slide AL evaluation kit (Schott) according to manufacturer's instructions. Functionalized slides were used immediately and incubated with beacon solution (20 µM in 1x PBS). Hybridization was monitored by confocal laser scanning microscopy (LSM 510, Carl Zeiss) with an inverted microscope (Axiovert 200 M, Carl Zeiss). Images were acquired using a plan-apochromat 20x objective (NA 0.75, Carl Zeiss). Cy3 was excited at a wavelength of 543 nm using a He/Ne laser (1 mW) at 80% maximal power output. Uncaging was performed with an Argon diode laser (20 mW) at 405 nm wavelength for 15 seconds (8 scans). Beacon solution for uncaging positive control samples was irradiated prior to hybridization as described in section **2.** Fluorescence images were processed with the Leica LAS AF software (Leica Microsystems).

5. Activation of caged beacon in human embryonic kidney cells

Human embryonic kidney cells (HEK293T) were cultured on poly-D-lysin coated glass cover slips in a 12-well culture dish containing Dulbecco´s Modified Eagle Medium (DMEM) supplemented with 10% FCS, 1% L-Glutamin and 1% Penicillin/Streptomycin. Cultures were kept in humidified atmosphere with 5% CO_2 at 37 °C for 3 d. Glass cover slips carrying HEK293T were transferred to a temperature controlled submersion-type recording chamber (at 37°C; Luigs and Neumann). The bath solution contained 126 mM NaCl, 2.5 mM KCl, 26 mM $NaHCO_3$, 1,25 mM NaH_2PO_4, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM glucose. Patch pipettes having a resistance of 6-10 MOhm were pulled from borosilicate glass capillary tubing (GC150TF-10; 1,5 mm outer diameter; Harvard Apparatus). The pipette solution contained 126 mM K-gluconate, 4 mM KCl, 4 mM ATP-Mg, 0.3 mM GTP-Na_2, 10 mM PO-Creatine, 10 mM HEPES (pH = 7.25 with KOH, 290 mOsm with sucrose) and 120 nM caged beacon MB2. Imaging was performed with an upright confocal microscope (Zeiss, Exciter; 543nm excitation, 560nm longpass emission filter, pinhole at 1 airy unit) equipped with a 40x water-immersion objective (0.9NA); after patching and keeping individual cells for 2 - 3 min in whole cell voltage mode (holding at -40mV). Single plane images were acquired at 0.2Hz. A Rapp Optoelectronic spot illumination device with attached UVILED light source was used for UV-irradiation (2 min).