Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Light-Inducible Molecular Beacons for Spatio-Temporally Highly Defined Activation

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Oligonucleotide Synthesis. Molecular beacons were synthesized on an Applied Biosystems Model (ABI) 392 synthesizer using 2'-OMe-RNA phosphoramidites. All standard cyanoethyl phosphoramidites were purchased from Link Technologies Ltd. and were employed according to their recommended coupling protocols. An internal amino modifier phosphoramidite was purchased from ChemGenes Corporation and introduced with 15 min coupling time. Cy3™ phosphoramidite was ordered from GE Healthcare Limited and employed with a 6 min coupling time. The synthesis was performed with BHQ2 (Black Hole Quencher®2) solid support (200 nmol scale) purchased from Link Technologies Ltd. After the synthesis the molecular beacons were cleaved from the solid support and deprotected with fresh ammonia (32% (v/v)) solution at 65 °C for two hours. Afterwards oligonucleotides were purified via reversed-phase HPLC. Nucleosil 100-5 C18 columns were always used with a gradient of 0.1 M TEAA (triethylammonium acetate, pH 7) and acetonitrile. The percentage of acetonitrile was increased from 0% to 47% within 50 min.

Deoxyguanosine phosphoramidite modified with NPP (2-(2-nitrophenyl)-propyl) as photocleavable group was synthesized as described previously[1] and used for solid phase synthesis with a 6 min coupling time.

The RNA target used for the *in vitro* testing of the respective molecular beacons was ordered from IBA GmbH, Göttingen, Germany.

After final purification, all oligonucleotides were characterized via mass spectroscopy (micrOTOF-Q by Bruker Corporation, negative mode).

Postsynthetic Dye Labelling. Atto550 was purchased from ATTO-TEC GmbH, Siegen, Germany as reactive NHS-ester. The labelling reaction of amino modified molecular beacons was performed in 0.1 M $NaHCO_3$ buffer (pH 8.5) for 90 min in the dark. The reaction mixture was subsequently purified via reversed-phase HPLC, as described above.

Fluorescence Measurement. For *in vitro* fluorescence measurements and beacon control experiments, a Tecan plate reader Infinite® 200 PRO was used with suitable 96-well plates from Cornwell (flat bottom, black, polystyrene). For each measurement, 25 pmol of the molecular beacon and 50 pmol of the target sequence were dissolved in PBS buffer (1x, pH 7.4) to a final volume of 100 µL.

Table S1. Excitation and emission wavelengths used for *in vitro* measurements of molecular beacons.

Dye	Excitation / nm	Emission / nm
Cy3	545	580
Atto550	550	580

***In vitro* Light-Activation of Molecular Beacons.** For light-induced removal of the NPP-group or cleavage of the commercially available photocleavable linker, the molecular beacon was irradiated for up to 3 min with 365 nm and 250 mW power using the LED "UVLED-365-250-SMD" from Roithner Lasertechnik. The irradiation experiments were always conducted in PBS buffer (1x, pH 7.4).
According to Figure S1 and S2, an illumination time of 1.5 min is sufficient to completely cleave 250 pmol of **MB3** and **MB4**.

DNase I Digestion of DNA Probes. For digestion tests DNA beacons **MB2'** and **MB3'** were synthesized, as described above. Deoxyribonuclease I (Amplification grade, Sigma Aldrich) was used. The reaction was carried out according to manufacturer's protocol and with the provided reaction buffer (50 pmol MB sample, 0.1 µL of the provided DNase solution, 37 °C). Directly after addition of the enzyme the fluorescence was measured with a Tecan plate reader Infinite® 200 PRO, as described above.

Preparation and Microinjection of *Chironomus tentans* Salivary Glands. Cultivation of the dipteran *C. tentans*, dissection of salivary gland and microinjection of oligonucleotides were performed, as described.[2] Dissected glands were transferred onto poly-L-Lysine coated cover slips for microscopic observation and were incubated in phosphate buffered saline (PBS) during microinjection and confocal imaging.

Quantification of Molecular Beacon Activation and Fluorescence Yield. To compare the activation efficiency of Cy3-labeled **MB2** and **MB3**, both substances were co-injected into *C. tentans* salivary gland cell nuclei with the ATTO550-labelled **MB4** as internal reference. The hybridization time for the beacons was determined to be in the order of 2-20s – depending on the amount of beacon used. ATTO 550 and Cy3 display different spectral properties, which could be used to separate the relative intensity contributions using spectrally resolved imaging with a LSM 510 META (Carl Zeiss Microscopy GmbH) equipped with a C-Apochromat 40X/1.2 W objective lens. In all experiments a HeNe laser emitting at 543 nm was used for fluorescence excitation. Fluorescence was separated from the excitation light using a HFT488/543 dichroic mirror, and detected using the META detector of the LSM. The collected fluorescence was split into 14 about 10 nm wide channels with their central wavelengths ranging from 558 nm to 697 nm.
Firstly, separate reference spectra of ATTO 550 and Cy3 labelled MBs were determined. To this end, either **MB3** or **MB4** were microinjected into nuclei of *C. tentans* salivary gland cells. Uncaging of the beacons was achieved by illumination of the glands with the high pressure mercury arc lamp of the fluorescence microscope using a DAPI filter set for 10 s (in optimization experiments with varying activation time this proved to be sufficient for virtually complete uncaging). Then, spectrally resolved images of the nuclei were acquired yielding a set of two times 14 images. Images showing the brightest five spectral channels were

shown in Fig. S4A for both dyes. In each of the image sets the region of the labeled BR transcription site was selected, and the average intensity of that region was calculated. This yielded coarse-grained spectra of both dyes within the intranuclear surroundings. Such spectra were measured in several different nuclei and averaged yielding the required reference spectra, $S_{Cy3}(\lambda_i)$ and $S_{Atto550}(\lambda_i)$ with $1 \leq i \leq 14$. The maxima of both reference spectra were defined as unity.

In a second step, a mixture of **MB2** or **MB3** was co-injected with **MB4** in a molar ratio of 1:1 into nuclei of explanted salivary gland cells. After uncaging spectral images of the BR transcription sites were acquired. Again, the mean spectral intensity of the BR transcription site pixels was determined as a function of the emission wavelength, $S_{mix}(\lambda_i)$. These spectra were decomposed by fitting them to a linear combination of the reference spectra,

$$S_{mix}(\lambda_i)=a_{Cy3}S_{Cy3}(\lambda_i)+a_{Atto550}S_{Atto550}(\lambda_i)$$

for each nucleus. The relative contributions a_{Cy3} and $a_{Atto550}$ were determined in 10 different nuclei for each beacon combination, and averaged. Still, it had to be considered that the excitation and detection probabilities of both dyes in the instrument were not identical. Taking the different maximal molar extinction coefficients, ε_M, the different excitation efficiency at 543 nm, η_{exc}, and the different fluorescence quantum yields, Q,, into account yielded corrected relative contributions, a'_{Cy3} and $a'_{Atto550}$.

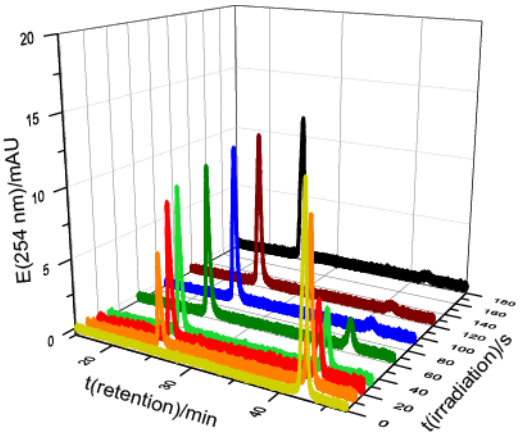

Figure S1. Uncaging of caged Q-Cy3 MB (**MB3**) followed via HPLC analysis (200 pmol, 365 nm, 250 mW, detection at 254 nm).

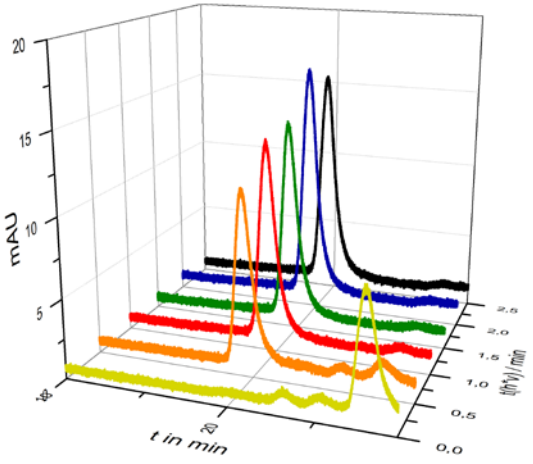

Figure S2. Photocleavage reaction of caged Q-Atto550 dye (**MB4**) followed via HPLC analysis detected at 254 nm. 150 pmol of the respective caged Q-Atto550 MB was used and illuminated for different times as described above.

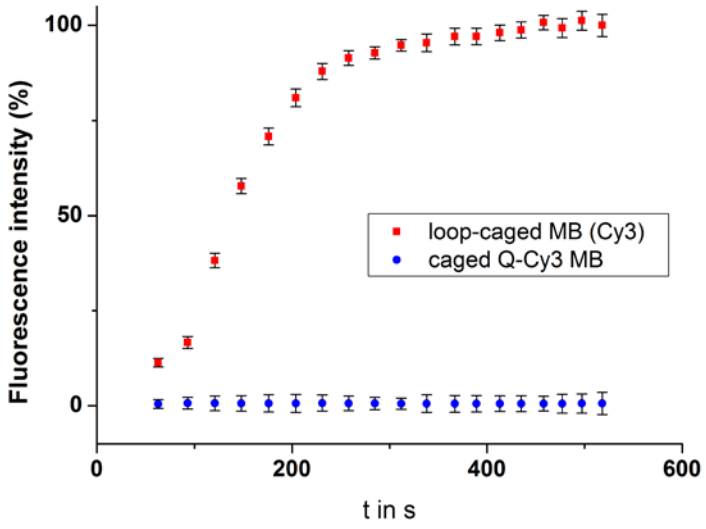

Figure S3. A DNA-based loop-caged molecular beacon (**MB2**) is rapidly degraded by DNase I. As a result, the fluorescent signal increases even in the absence of target and without light-activation. A caged Q-dye MB does not show an increase in fluorescence, false positive results are reduced. The linkage between fluorophore and quencher of the MB is not cleaved by nucleases. The fluorescence is based relatively to the fluorescence of an equal amount of Cy3™.

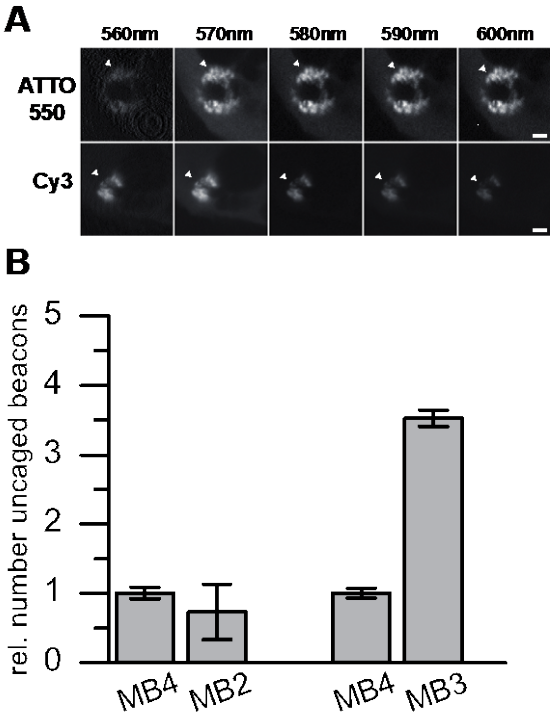

Figure S4. Quantification of signal increase of MB2 and MB3. (A) Detection of Cy3- and ATTO550-labeled molecular beacons, **MB3** and **MB4** that label the BR2 mRNPs inside of *C. tentans* salivary gland cell nuclei. In the shown images, **MB3** and **MB4** were injected into different cell nuclei. The panels show intranuclear regions containing the BR2 transcription sites with their characteristic donut shape in confocal sections as a function of the emission wavelength after illumination for 10 s with UV light. The images were obtained using the spectral detector an LSM 510 META (Zeiss, Jena, Germany). The different spectral emission behaviour of the dyes allowed to clearly descriminate Cy3 and Atto550. Scale bar, 10 µm. (B) The amounts of **MB2** and **MB3** in living *C. tentans* cell nuclei upon uncaging were determined relative to that of **MB4** as described in the experimental section. Thus, the signal intensity ratio of **MB4** after uncaging was unity by definition, and the signal increase of **MB2** respectively **MB3** was determined in relation to that of **MB4**. The figure shows that the signal increase of **MB3** compared to that of **MB2** was about 5-fold.

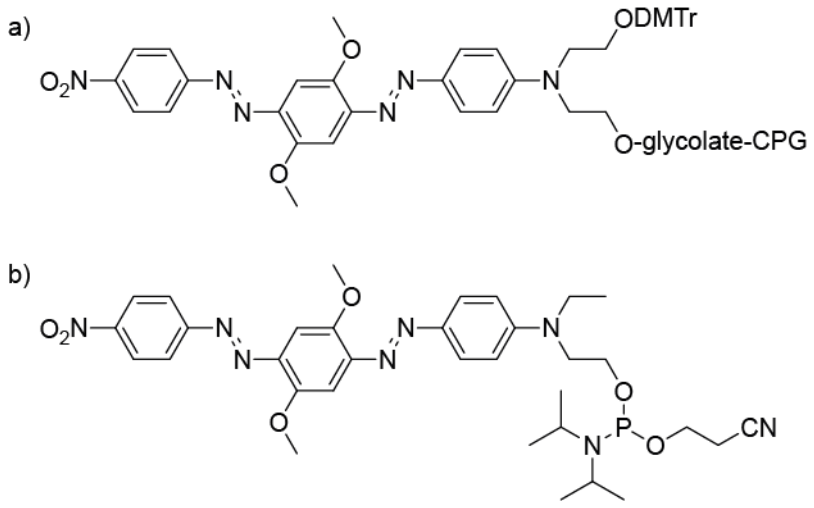

Figure S5. Molecular structures of the commercially available units containing the quencher BHQ2 which were used in these studies: a) BHQ2-containing solid support used for the 3'-modification of the molecular beacons (Linktech, 3'-BHQ2 CPG 1000). b) phosphoramidite unit used for the 5'-modification of the molecular beacons (Linktech, 5'-BHQ2-CE phosphoramidite).

References for the supporting information.

1. G. Mayer, L. Kröck, V. Mikat, M. Engeser, and A. Heckel, *ChemBioChem*, 2005, **6**, 1966–1970.
2. T. Kaminski, J.-H. Spille, J. P. Siebrasse, and U. Kubitscheck, *Methods Mol. Biol. (N. Y.)*, 2013, in press.