Caged Molecular Beacons: Controlling Nucleic Acid Hybridization with Light

Chunming Wang,[a †] Zhi Zhu,[b †] Yanling Song,[a] Hui Lin,[a] Chaoyong James Yang [*a] and Weihong Tan[*b]

a State Key Laboratory of Physical Chemistry of Solid Surfaces, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005 (China) E-mail: cyyang@xmu.edu.cn

b Department of Chemistry and Department of Physiology and Functional Genomics, University of Florida, Gainesville, Florida 32611-7200 (USA) E-mail: tan@chem.ufl.edu

1. General information and methods ----------S2

2 Reaction Rate of the Photocleavage Reaction ----------S3

3 MALDI-MS analysis---------- ----------S4

4 PAGE analysis ----------S5

5 Thermal stability----------S7

6 Synthesis of click reaction-based cMBs ----------S7

7 Hybridization of click reaction-based cMBs ----------S8

8 PAGE analysis of click reaction-based cMBs----------S8

9. References----------S9

Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 201X

Caged Molecular Beacons:Control Nucleic Acid Hybridization with Light

Chunming Wang,[a †] Zhi Zhu,[b †] Yanling Song,[a] Hui Lin,[a] Chaoyong James Yang [*a] and Weihong Tan[*b]

[a] *State Key Laboratory of Physical Chemistry of Solid Surfaces, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005 (China) E-mail: cyyang@xmu.edu.cn*

[b] *Center for Research at Bio/nano Interface, Department of Chemistry and Department of Physiology and Functional Genomics, Shands Cancer Center, UF Genetics Institue and McKnight Brain Institute, University of Florida, Gainesville, Florida 32611-7200 (USA) E-mail: tan@chem.ufl.edu*

1. General Information and Methods

NeutrAvidin was purchased from Thermo Fisher Scientific Inc. (Rockford, USA) and used without further purification. Tris [(1-benzyl-1H-1, 2, 3-triazol-4-yl) methyl] amine (TBTA) and Stains-All were purchased from Sigma-Aldrich Inc. Molecular beacons were synthesized on an ABI 3400 DNA synthesizer, and the complementary DNA was synthesized on a PolyGen DNA synthesizer. The reagents for DNA synthesis were purchased from Proligo (Sigma-Aldrich Inc.), Glen Research (Sterling, VA, USA) and ChemGenes (Wilmington, MA, USA). All of the oligos were purified by an Agilent (Santa Clara, CA, USA) 1100 series HPLC system on a reverse-phase C18 column. All DNA sequences are listed in Table S1.

The UV irradiation experiment was performed using a Lightningcure Series LC8 UV spotlight source from Hamamatsu Photonics K.K. (Japan) with a wavelength of

365 nm. For the kinetics study of photocleavage reaction experiment, 5 μL of MBs with the concentration of 100 μM were pipetted onto a quartz glass coverslip and exposed to UV light. The illumination time was recorded with a timer. After that, the samples were collected again and analyzed by HPLC.

Fluorescence measurements were carried out on a RF-5301-PC Fluorescence Spectrophotometer (Shimadzu, Japan). In time scanning mode, excitation and emission wavelengths were set at 490 and 515 nm, respectively, with the bandwidth of 5 nm. The emission spectra were obtained by exciting the samples at 490 nm and scanning the emission from 500 to 600 nm at 1 nm intervals.

Table S1. Detailed sequences of different molecular beacons.

Molecular beacons	Sequences
PC0	5'- Biotin-PEG-FAM-CCT AGC TCT AAA TCA CTA TGG TCG CGC TAG G-DABCYL-PEG-Biotin-3'
PC1	5'- Biotin-PC-linker-PEG-FAM-CCT AGC TCT AAA TCA CTA TGG TCG CGC TAG G- DABCYL -PEG-Biotin-3'
PC2	5'- Biotin-PC-linker-PEG-FAM-CCT AGC TCT AAA TCA CTA TGG TCG CGC TAG G- DABCYL -PEG-PC-linker-Biotin-3'
Click MB	5'-alkynyl-PC-linker-FAM-CCT AGC TCT AAA TCA CTA TCG CGC TAG G- DABCYL -azido-3'
Normal MB	5'-FAM-CCT AGC TCT AAA TCA CTA TGG TCG CGC TAG G-DABCYL -3'

2．Reaction Rate of the Photocleavage Reaction

In order to find out how fast the photoreaction was going, four identical samples of PC1 were subjected to UV irradiation for about 0.5 s, 1.0s, 1.5 s and 2.0 s respectively. After that, the samples were analyzed by HPLC. Since there was only one reactant, we assumed that the photocleavage is a first order reaction. The intensity of the probe at 0 s was set as 100 and the others were set proportionally. The data

were then fitted to a first order reaction expression by linear regression. As Fig. 2 shows, the half time could be easily calculated according to the equation of the fitted line (while $t_{1/2} = \ln 2/k$), which revealed that half of the MB had been cleaved in about 0.6 s. We concluded that the photoreaction processed very fast and finished within seconds.

3. MALDI-MS analysis

MALDI-MS was used to confirm the successful synthesis of cMB and successful cleavage of cMB after UV irradiation. Briefly, 1μL of matrix 3-Hydroxypicolinic Acid (3-HPA) was first pipetted onto the plate, and then 1μL of samples were pipetted and mixed thoroughly with the matrix. After the solvent evaporated, the plate was sent for MS analysis. The molecular weight of PC1 was calculated to be 12353Da, and the measured MW was 12356Da, suggesting successful synthesis of the probe. More importantly, after photocleavage, the calculated molecular weight of the probe should be 11751Da, and MALDI-MS analysis result gave a molecular weight of 11754Da, confirming that the photocleavage reaction took place as expected.

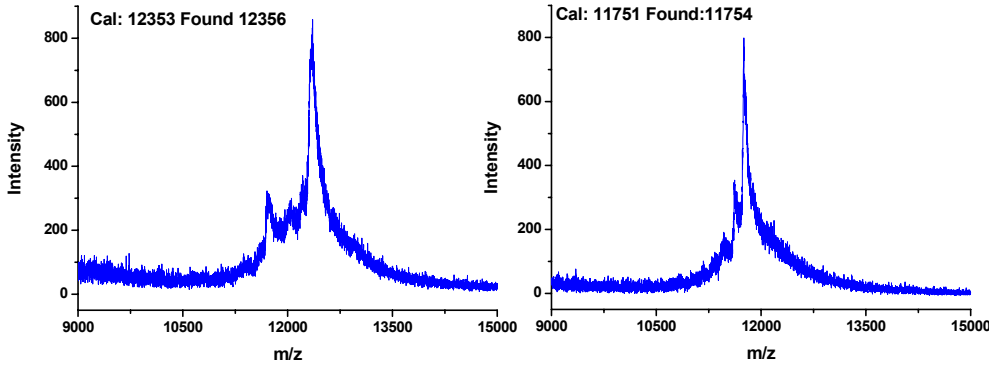

Fig. S1 MALDI-MS characterization of PC1 before (left) and after UV irradiation (right).

4. PAGE Analysis

Polyacrylamide gel electrophoresis (PAGE) is a technique frequently used for protein and small nucleic acid separation. Here we used PAGE to confirm that there were interactions between MB and NeutrAvidin (both in graphic and text below, NeutrAvidin is shortened to "avidin").

In Fig. S2 (a), we tried to find out the best dose of avidin, in order to caged all the free PC1 MBs. Because size of the avidin (NeutrAvidin: 60 kD) is big, molecular beacons would not penetrate into the gel if bound to avidin. Only unbound MBs can be seen in the gel. For a normal MB (lane 1-3), neither the addition of avidin nor the irradiation by UV light would affect its migration rate. For lane 4-9, different equivalents of avidin were incubated with PC1. At low concentration of avidin, not all the probes were bound to protein. There were still some free probes. As more avidin was added, more probes would bind to avidin to form a DNA-protein complex and as a result the free MB band in the gel became weaker. With 2 equivalent of avidin, no visible band could be seen (lane 8), which indicated that all the probes had bound to avidin. For PC1, there is only one PC linker, which means that the probe will still bind to avidin even after UV activation. As a result, the probe couldn't migrate into gel even after UV exposure.

In Fig. S2 (b), two control sequence PC0 and PC2 were introduced to prove that this type of MB could indeed be caged by interacting with avidin and activated by UV light. PC0 has no PC linker in the sequence, while PC2 has a PC linker at either end of its sequence. After exposing to UV light, for PC2, both of its PC-linkers would be

cut, thereby liberating the probe from avidin so that the cleaved probe can migrate into the gel. As shown in Fig. S2b lane 5, no free PC2 band was observed for PC2 avidin sample. However, after exposure to UV, a strong band showed up (lane 6), which suggested the probe could be activated by UV exposure. Because of the decrease in molecular weight, cleaved PC2 migrated faster than the pristine probe (lane 6). In contrast, PC0, which has no PC-linkers, did not respond to UV light (lane 8 and 9).

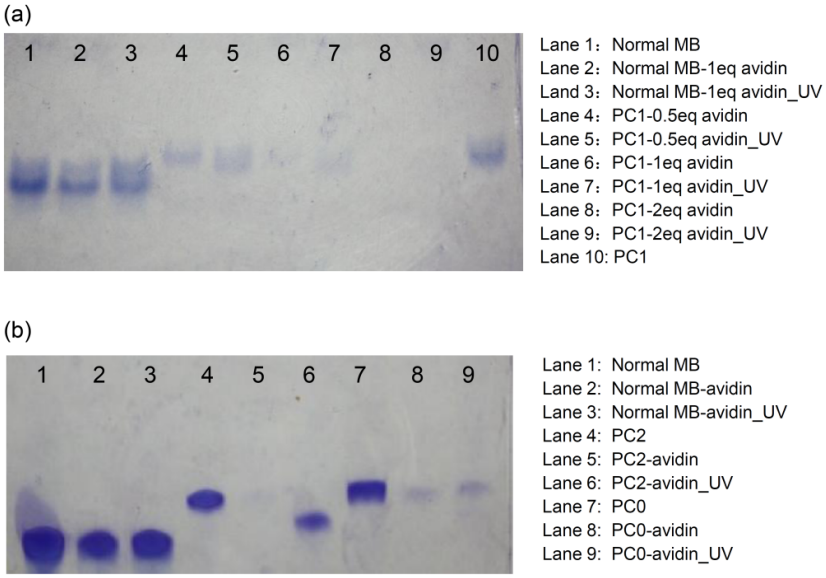

Fig. S2 PAGE analysis of different MBs (gel concentration: 20%). Electrophoresis was carried out in 1×TBE (pH 8.3) buffer at a constant power of 1W for about 1.5 h. After Stains-All staining and destaining, a picture of the gel was taken by a Canon EOS 450D Digital Camera.

These results proved that cMBs could be fully caged by interacting with avidin and completely activated by UV light illumination.

5. Thermal stability

Melting curve analysis is commonly employed to assess the structural stability of MBs. Herein, the melting curves of a caged MB and a normal MB were recorded respectively (Fig. S3). It was found that the caged MB was much more stable under high temperature condition, compared to normal MB. The caged MB showed only a little fluorescence enhancement upon being heated to 90℃. Once it was activated by UV light, however ,it became sensitive to the change of temperature (left). In contrast, UV irradiation did not change the stability of a normal MB. The results again confirmed the successfully caging and uncaging of cMBs as expected.

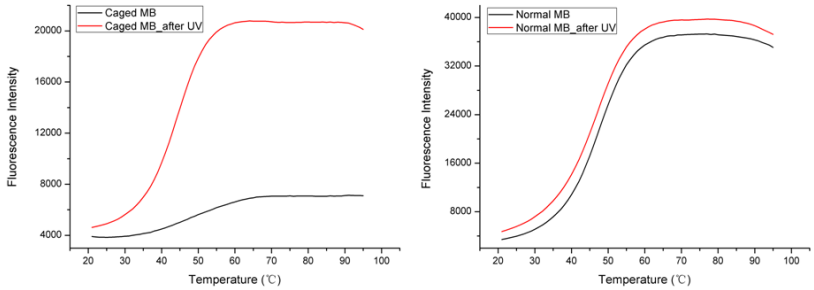

Fig. S3 Thermal profile comparison of caged MB with normal MB. 100 nM of MBs in PBS buffer (137 mM NaCl, pH 7.4). The data were acquired on an ABI StepOne RT-PCR system with the temperature ranging from 20 ℃ to 95 ℃ at 1 ℃ interval.

6. Synthesis of click reaction-based cMBs

The synthesis began on the automated DNA synthesizer with the amino-on-CPG (Proligo), and ended by coupling to hexynyl phosphoramidite. After normal cleavage and deprotection, the probe was purified by RP-HPLC. Then the MB was incubated

with azido butyrate NHS ester in sodium bicarbonate buffer (pH 8.75) for 4 h to yield an azido-terminated MB.[1] After a second round of HPLC, the MB was used for click reaction to produce the caged MB in the presence of CuI/TBTA (concentration ratio =1:9).

7. Hybridization of click reaction-based cMBs

To prove the click reaction based cMBs can work as we proposed, fluorescence response of cMB to its target DNA was studied. At caged state, there were no or few MB molecules that partially hybridized to target DNA. As a result, only a weak fluorescence was observed. While at the activated state, cMBs could hybridize freely with cDNA, thus gives out strong fluorescence (Fig. S4).

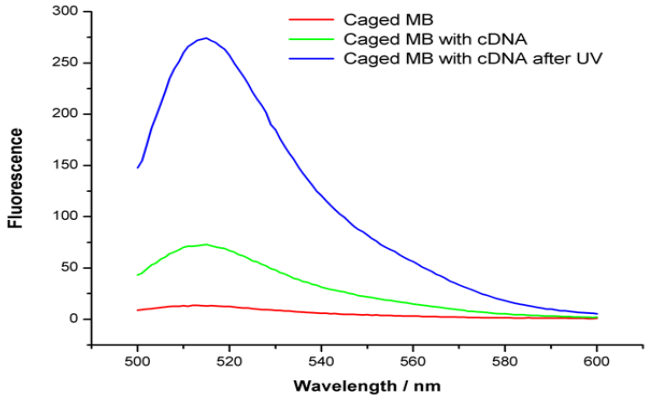

Fig. S4 Fluorescence emission scanning of click reaction-based cMBs under different conditions. 100 nM of MBs in 20 mM Tris-HCl buffer (140 mM NaCl, pH 7.4), cDNA concentration = 500 nM.

8. PAGE analysis of click reaction-based cMBs

Pre-caged MB (before click reaction was performed) behaved just like a normal MB in that it could hybridize to its cDNA to form a complex (lane 2 and 3). However, when it was caged (after the click reaction was performed), it migrated much faster. Even the addition of cDNA did not result in much change to its migration rate (lane 4

and 5). Only when it was activated by UV light, could it again behave like the pristine MB (lane 6 and 7).

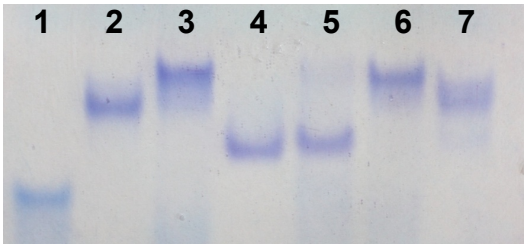

Fig. S5 Native PAGE analysis of click MB. (gel concentration: 20%). Electrophoresis was carried out in 1×TBE (pH 8.3) buffer at a constant power of 1W for about 1.5 h. After Stains-All staining and destaining, a picture of the gel was taken by a Canon EOS 450D Digital Camera.

9. References

S1. R.Kumar, A.EI-Sagheer, J.Tumpane, P.Lincoln, L.M.Wilhelmsson, T.Brown, *J. Am. Chem. Soc.* 2007, **129**, 6859-6864.