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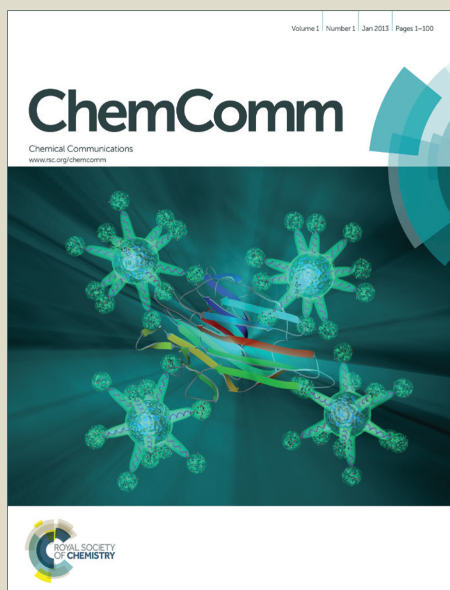

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COMMUNICATION

Cite this: DOI: 10.1039/x0xx00000x

Self-assembled conformational switch: host-guest stabilized triple stem molecular beacon *via* photoactivated and thermal regeneration mode

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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We present a novel strategy for construction of conformational switch of molecular beacon based on the combination of nucleic acid (DNA) self-assembly and reversible host-guest inclusion interaction. With the functionalized probe, the nucleic acid hybridization process can be easily controlled with a photoactivated and thermal regeneration mode.

Nowadays, there is an ever growing interest in controlling the conformations and functions of molecules by external stimuli. Among numerous effective external stimuli, such as pH,[1] redox,[2] light,[3] and electric field,[4] light is especially of interest because it works rapidly, remotely, and cleanly with relatively high spatial and temporal precision. Recently, photocaging strategy has emerged as an innovative solution for the spatiotemporal study of biomolecular functions, such as nucleic acid hybridization,[5] gene expression,[6] enzymatic activity,[7] etc. Although the concept of photoactive groups has been introduced in these studies, the functionalized molecules actually underwent irreversible photochemistry process. In contrast, reversibly photoactive groups have been incorporated in a range of molecules, and produced cycling between active and inactive states for modulating the physical/chemical properties of the molecules.[8] Azobenzene moieties, as a kind of switchable chemical groups for the photoactivation of biomolecules, have been attached in nucleic acids[9] or peptides[10] to construct conformational switches for *in vitro*[11] and *in vivo*[12] applications.

Usually, it was complicated issue that the covalent attachment of two or more functional groups to key sites of biomolecules was essential for the above conformational switches. Nucleic acid (DNA) self-assembly based on spontaneous hybridization between complementary strands has shown its practicability to be a highly effective mean of acquiring functional DNA probes without complex modification.[1,13]

Here, we have designed a self-assembled conformational switch *via* photoactivated and thermal regeneration mode (Fig. 1), inspired by the photo/thermal-responsive β-cyclodextrin/azobenzene (β-CD/Az) host-guest complex[14] and nucleic acid (DNA) self-assembly for functional DNA probes. The conformational switch is regulated by host-guest recognition interaction that can facilitate the reversible cycling between locked /hybridization state by virtue of its own unique structural architecture. In addition, the functionalized probe is designed as a triple stem molecular beacon comprised of three DNA strands based on spontaneous hybridization, thus avoiding multiplex modification on a single strand of DNA.

The construction and operation of a triple-stem molecular beacon (TMB1) is presented in Fig. 1. TMB1 consists of three DNA strands:[15] a long DNA strand labeled with fluorophore/quencher (F/Q) pair (hairpin DNA: 5'-CCT GCC ACG CTC CG CT^{BHQ1}G CGA GCC ACC AAA TAT GAT ATG CTC GCT^{FAM} CTC GCA CCG TCC ACC-3'), and two short DNA strands modified with azobenzene (Az-DNA: 5'-Azobenzene-TGG TGG ACG GTG CGA G-3') and β-CD (CD-DNA: 5'-GCG GAG CGT GGC AGG-β-cyclodextrin-3'), respectively (Table S1 and Fig. S2 in ESI). Az-DNA and CD-DNA are complementary to the 3' and 5' domain of hairpin DNA, respectively. Thus, the main components of TMB1 include the hairpin backbone (stem-1, stem-2, stem-3), β-CD/Az moiety, and the fluorophore/quencher (F/Q) pair with a convenient 5 nm Förster radius for signaling conformational change.[16] In the locked state, due to the stabilization effect of internal hybridization in stem-1 and the formation of stable host-guest inclusion complex (β-CD/*trans*-Az),[14] TMB1 is inactive and emits weak fluorescence even in the presence of targets. However, TMB1 can be activated to make the conformational switch changing from the locked state to the hybridization state by UV irradiation (365nm), then emit strong fluorescence of FAM, due to the weak inclusion interaction between β-CD and *cis*-Az and the formation of target-probe

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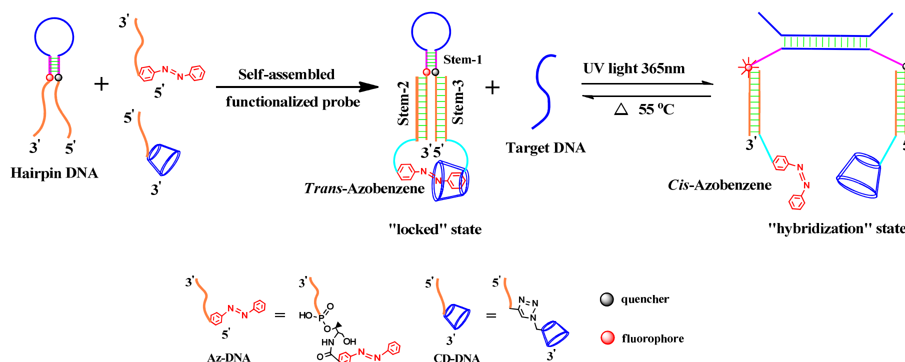

Fig. 1 Schematic representation of reversible modulation of the conformational switch for nucleic acid hybridization, based on triple stem molecular beacon and host-guest complex between azobenzene and β-cyclodextrin

duplex. In addition, TMB1 can reversibly revert to the locked state by thermal regeneration mode (55 ^{O}C), since the heating can induce the release of target from duplex and the *cis*-to-*trans* conversion of the Az moieties, and then the stable host-guest inclusion complex of β-CD/Az are formed again. As such, TMB1 allows us to execute the function of the probe on a time frame and spatial scale.

To demonstrate that TMB1 could be activated by photo-regulation as we expected, fluorescence measurements of the probe were performed. TMB2, another triple stem molecular beacon without β-CD, was used as a control (Table. S1 in ESI). As shown in Fig. 2, compared to TMB2, the locked TMB1 showed lower background fluorescence, resulting from the host-guest (β-CD/*trans*-Az) interaction which stabilized hairpin structure to achieve higher fluorescence resonance energy transfer efficiency between F/Q pair. When the target DNA was added, the locked TMB1 still showed low fluorescence without irradiation, the slight change of fluorescence intensity was most likely a result of partial binding of target DNA to TMB1. However, TMB2 showed a notable fluorescence increase. As we expected, TMB1 showed a significant fluorescence increase only in the presence of targets by UV irradiation. Therefore, the fluorescence increase of TMB1 was the result of the photo-dissociation of the β-CD/*trans*-Az complexes by UV light-induced *trans*-to-*cis* isomerization of Az moieties. We only observed 7.9 % recovery for TMB1 solution which was

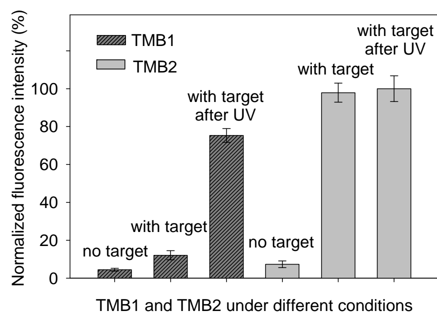

Fig. 2 The fluorescence response histogram of TMB1 and TMB2 under different conditions at 25°C. 100 nM TMB1 or TMB2, 400 nM target DNA in 20 mM Tris-HCl buffer (pH 7.4, 140 mM NaCl, 5 mM $MgCl_2$). Fluorescence intensity was normalized to a percentage of the maximum signal coming from 100 nM TMB2 hybridized with 400 nM target DNA after UV irradiation for 30min at 25°C. The fluorescence spectra were shown in Fig. S3 of ESI.

hybridized with target DNA without UV irradiation, but the recovery increased to 74% after UV irradiation for 30 min (Formula 1 in ESI). These results strongly suggested that TMB1 could be activated by UV light to open the conformational switch for nucleic acid hybridization. By adding competitive guest molecules (amantadine) to TMB1 solution instead of UV irradiation (Fig. S4 in ESI), the significantly increased fluorescence demonstrated that the conformational switch of TMB1 was actually regulated by β-CD/Az host-guest interaction.

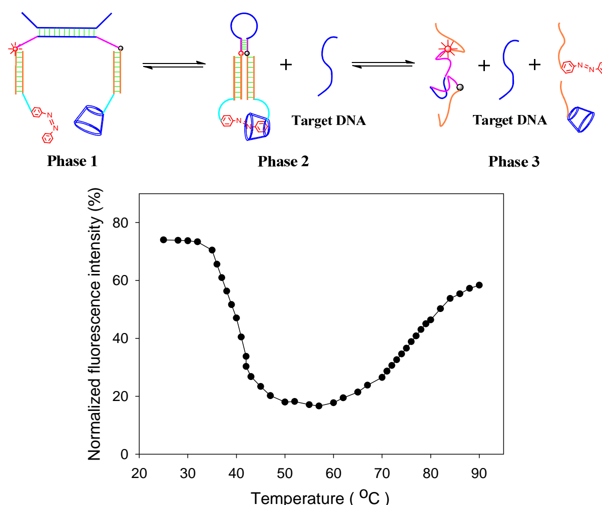

Fig. 3 The effect of temperature on the probe TMB1. Up: The proposed phase transitions of TMB1 in the presence of targets at different temperatures; Down: Melting curve of 100 nM TMB1 hybridized with 400 nM targets. Normalization of the fluorescence using the same condition as Fig. 2.

In order to demonstrate whether the host-guest stabilized TMB1 could be regenerated by thermal effect, fluorescence of the probes incubated with targets were monitored at different temperatures. TMB1 was assumed to exist in three different phases in the presence of targets:[17] Phase 1, as a target-probe duplex; Phase 2, as a native configuration; and Phase 3, as a random coil (Fig. 3, up). At lower temperature, TMB1 could hybridize with the target (Phase 1, T_m=40.2°C) after UV irradiation, which led to significantly increased fluorescence (Fig. 3, down). As the temperature increased, the target-probe duplex was destabilized, and the released probe gradually refolded into its native structure (Phase 2, T_m=75.3°C), which resulted in significantly diminished fluorescence. As the temperature increased further, the folded probe molten into random

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coils (Phase 3), in which fluorescence increase was observed. The system emitted the lowest fluorescence at the temperature of 55°C. Such a low fluorescence could be explained in three aspects. First, target-probe duplex was destabilized and the probe was released at this temperature. Second, the strong interaction between Az-DNA/CD-DNA and hairpin DNA could be achieved by having a high GC content in stem2 and stem3 which had a calculated T_m of 63.9/67 °C, respectively. So stem1, stem2 and stem3 could make the space proximity of β-CD and azobenzene. Third, the *cis*-azobenzene thermally reverted to the initial *trans*-isomer,[18] which easily formed a stable inclusion complex with β-CD in the lock state. Therefore, 55°C was selected for thermal regeneration of TMB1.

To further study kinetics of the conformation switch, the fluorescence of TMB1 incubated with targets was monitored under various conditions. As shown in Fig. S5 of ESI, the photoactive significant signal changes of the probe could be observed in 5 min and the signal tended to a stable value in 10 min. Similar tendency of thermal regeneration was observed. To show the maximum response signal of the conformational switch, we presented the results of signal changes using the 30 min protocol for photoactivation and thermal regeneration, respectively.

At the same time, fluorescence responses of the system for multiple cycles were investigated, as shown in Fig. 4. Through photoactivated and thermal regeneration mode, TMB1 could be used for reversible control of DNA hybridization as we expected.

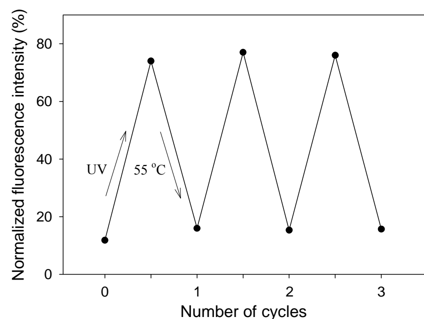

Fig. 4 Reversible light/thermal-response of TMB1 with target DNA. 100 nM TMB1, 400 nM target DNA in 20 mM Tris-HCl buffer. The temperature of UV light irradiation was 25°C. Normalization of the fluorescence using the same condition as Fig. 2.

Conclusions

In conclusion, we have demonstrated a novel strategy for the construction of conformational switch of molecular beacon based on the combination of nucleic acid (DNA) self-assembly and reversible host-guest inclusion interaction. With this strategy, through photoactivation and thermal regeneration of TMB1, we are able to reversibly regulate the molecular probe function for nucleic acid hybridization. In addition, self-assembled functional modification of the non-recognition region in the probe can avoid multiplex markers on a single strand of DNA, and avoid impact on the binding of the probe/target complex. Furthermore, we believe that this strategy would add a new concept to the design of functionalized nucleic acid probes with modulatable structure and function *via* external stimuli.

This work was supported in part by the Natural Science Foundation of China (21175035, 21190040, 21205033), International Science & Technology Cooperation Program of China (2010DFB30300).

Notes and references

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Electronic Supplementary Information (ESI) available: experimental details, synthesis and characterization of CD-DNA, photoactive assay, regulation of TMB1 by guest molecules and kinetics study See DOI: 10.1039/c000000x/

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