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Switchable β-cyclodextrin (β-CD) induced hydrolysis of *m-tert*-butylphenyl acetate is demonstrated in the presence of supramolecular β-CD/adamantane oligonucleotide scaffolds. In one system the duplex between a β-CD-functionalized nucleic acid and an adamantane-nucleic acid leads to a switchable catalytic system. In a second system a β-CD/adamantane duplex is cooperatively-generated by K^+-stabilized G-quadruplex units. The binding of hemin to second system yields a bifunctional DNA scaffold of alternate catalytic functions.

Reactions assisted by DNA templated structures attract substantial research interest as a means to increase the rates of processes (due to the enhanced local concentration of the reactants),[1] control of regio-selectivity,[2] induce chiroselectivitiy[3] and to develop synthetic means for the ligation oligonucleotides.[4] For example, Roelfes et al. investigated in asymmetric Diels–Alder reactions the role of the second coordination sphere around the metal centre in a DNA hybrid catalysts containing a covalently anchored Cu(II) complex using a modular strategy for the screening of specific DNA sequences.[5] In addition, Mokhir et al. reported a naphthaleneimide–alkyne profluorophore which is used as a reporter for the formation of a triazole product. In that study oligonucleotides functionalized with the alkyne profluorophore and an azide at the 3' and 5' ends, respectively, led to at reaction proceeding at template concentrations as low as 20 nM.[6]

Despite the progress in the application of supramolecular systems for DNA-templated synthesis,[7] the use of such systems for switchable catalysis is, to the best of our knowledge, unprecedented. The advances in the development of DNA machines and DNA switching devices were recently implemented to develop switchable enzymes,[8] DNAzymes[9] and even enzyme/DNAzyme cascades.[10] For example, the DNA-fuel-driven strand displacement of an interlocked DNA catenane nanostructure was implemented to switch the nanostructure between "ON" and "OFF" catalytic DNAzyme states.[11] Similarly, the closure and opening of a DNA tweezers structure modified at its ends with the enzymes glucose oxidase (GOx) and horseradish peroxidase (HRP), led to the "ON" and "OFF" activation of the GOx/HRP enzymatic cascade by means of the K^+ ion-stimulated formation of a G-quadruplex, and its separation by 18-crown-6-ether.[12]

In the present study, we report on the integration of β-cyclodextrin (β-CD) with a DNA scaffold and the "ON"/"OFF" switchable control of the catalytic functions of β-CD by means of this DNA scaffold. We decided to use as a model system the hydrolysis of *m-tert*-butylphenyl acetate catalyzed by β-cyclodextrin because it is a well-characterized reaction.[13] We further demonstrate the cooperative formation of a K^+ ion-stabilized G-quadruplex DNA scaffold, that includes the β-CD catalyst system, and the reversible separation of the G-quadruplex by means of 18-crown-6-ether, leading to the switchable DNA-scaffold-controlled "ON" and "OFF" activation of the β-CD catalytic functions. We also highlight that the incorporation of hemin into the G-quadruplex unit, that cooperatively stabilizes the β-CD DNA scaffold, results in a functional DNA scaffold of dual switchable catalytic functions. Namely, the supramolecular DNA system leads to the switched-on hemin/G-quadruplex when the β-CD catalytic properties are switched-off, whereas upon switching-on the catalytic functions of β-CD, the catalytic features of the β-CD are switched off.

The supramolecular switchable DNA system is based on well-established principles of β-CD chemistry: (i) The β-CD binds the *m-tert*-butylphenyl moiety and acts as a catalyst for the hydrolysis of *m-tert*-butylphenyl acetate (**3**) with the concomitant acylation of the β-CD. (ii) Adamantane also binds

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Electronic Supplementary Information (ESI) available: [Instrumentation, materials and general experimental procedures, DNA sequences, synthesis of (**1**), (**2**), (**6**), and (**7**)].
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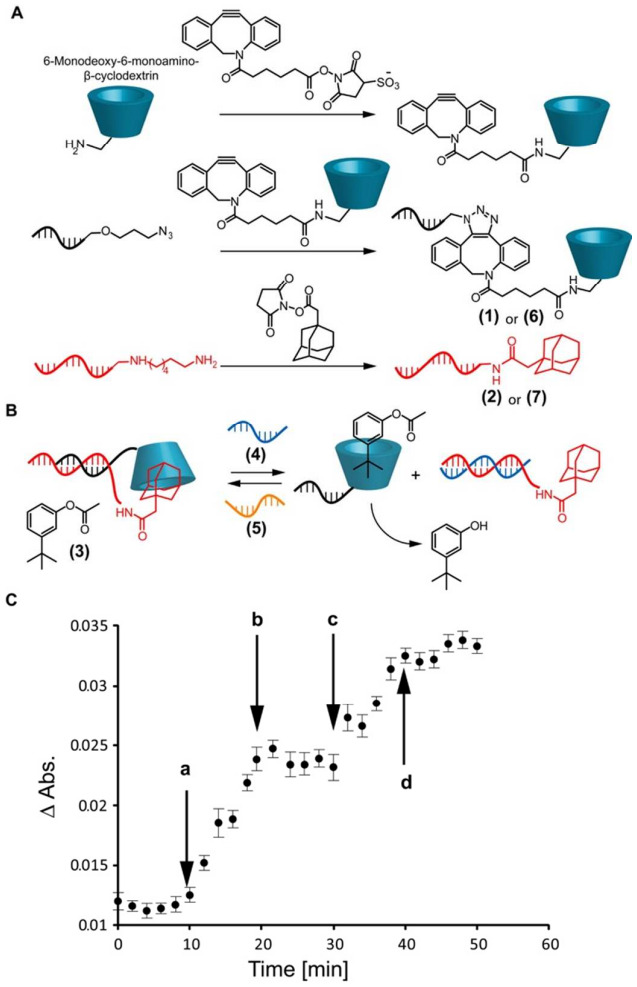

Fig. 1. (A) Synthesis of β-CD-functionalized nucleic acids (**1** & **6**) and of adamantane-modified nucleic acids (**2** & **7**). (B) Schematic representation of the β-CD catalyzed hydrolysis of *m-tert*-butylphenyl acetate (**3**) using the (**1**)/(**2**)-β-CD/adamantane duplex structure as an "ON"/"OFF" regulating scaffold, using appropriate fuel (**4**), and anti-fuel (**5**), DNA strands. (C) Time-dependent absorbance changes observed upon hydrolysis of (**3**) in the presence of (**1**)/(**2**) system and (**4**) and (**5**) as fuel and anti-fuel triggers. The experiment is initiated by subjecting the substrate (**3**) to the (**1**)/(**2**) scaffold. At point (a) the fuel strand (**4**) is added to the system. At point (b) the antifuel strand (**5**) is added to the system. Points (c) and (d) represent the times where the fuel strand (**4**) and anti-fuel strand (**5**) are re-added sequentially. Experiments were recorded using a buffer solution HCO_3^-/CO_3^{2-} (10 mM, pH=9.2), oligonucleotides (**1**) and (**2**) 10 μM, and *m-tert*-butylphenyl acetate (**3**) 100 μM, $MgCl_2$ 5 mM, at 30 °C. Error bars derived from N= 3 experiments. The hydrolysis of (**3**) was followed at λ= 275 nm. In order to obtain the net absorbance changes, ΔAbs. as a result of hydrolysis of (**3**) the background absorbance of the added strands (**4**) and (**5**) was subtracted, at λ= 275 nm, from the gross absorbance changes occurring in the system.

to β-CD and might act as inhibitor for the β-CD cavity, but at micromolar concentrations most of the β-CD is free in solution (K_a=10^5 M^{-1}).[14] The β-CD–oligonucleotide conjugates (**1**) and (**6**) were prepared by reaction of azide-modified oligonucleotides with dibenzocyclooctyne-sulfo-N-hydroxysuccinimidyl ester and 6-monodeoxy-6-monoamino-β-cyclodextrin. The adamantane-modified oligonucleotides (**2**) and (**7**) were synthesized by reacting amino functionalized oligonucleotides with N-hydroxysuccinimide adamantane acetate. The typical yield of the reactions determined by HPLC was 60%. The nucleic acids (**1**) and (**2**) include complementary domains, and thus the hybridization between (**1**) and (**2**) increases the local concentration of the adamantane units in proximity to the β-CD units leading to a tight complex between the adamantine units and the β-CD receptor sites. Figure 1(B) depicts the cyclic switchable ON/OFF catalyzed hydrolysis of (**3**) in the presence of the β-CD-nucleic acid scaffold. In the presence of the (**1**)/(**2**) duplex structure, the catalyzed hydrolysis of (**3**) is switched off. The duplex (**1**)/(**2**) does not reveal catalytic functions, due to the blocking of the β-CD units by the adamantane inhibitor. Treatment of the duplex (**1**)/(**2**) with the fuel strand, (**4**), results in the displacement of (**2**) and the formation of the energetically-stabilized duplex (**2**)/(**4**). The free DNA strand (**1**) then acts as catalyst for the hydrolysis of (**3**), Figure 1(B), point (b). Further addition of (**5**) displaces the duplex (**2**)/(**4**) while forming the stable "waste" duplex (**4**)/(**5**). The released (**2**) re-hybridizes with (**1**), resulting in the switching off of the catalytic functions of (**1**), Figure 1(B), point (c). By the cyclic addition of (**4**) and (**5**) to the system, the catalytic functions of the β-CD-modified strand (**1**), towards hydrolysis of (**3**), are switched between "ON" and "OFF" states, respectively. The catalytic functions of (**1**) towards the hydrolysis of (**3**) were characterized separately, Figure S1 supporting information. The derived k_{cat} =8 X 10^{-6} s^{-1} was calculated for the catalyzed hydrolysis of (**3**) by (**1**).

A second switchable DNA scaffold is made of a β-CD-functionalized nucleic acid, (**6**), which includes two domains I and II, and an adamantane-modified nucleic acid, (**7**) containing the domains III and IV. Domains I and III are complementary to each other, but the resulting duplex (7 bases) is unstable at room temperature. The domains II and IV are G-rich, and under appropriate conditions can self-assemble into a G-quadruplex structure. In the presence of K^+ ions the inter-strand G-quadruplex is stabilized and in turn cooperatively stabilizes the formation of the (**6**)/(**7**) duplex. Under these conditions, adamantane binds to the β-CD, and the catalytic functions of β-CD toward hydrolysis of (**3**) are switched off. Addition of 18-crown-6-ether to the system, eliminates the K^+ ions from the G-quadruplex, resulting in the separation of the G-rich subunits and the dissociation of duplex (**6**)/(**7**). The release of (**7**) yields the free β-CD-modified strand (**6**) that catalyzes the hydrolysis of (**3**). A subsequent addition of K^+ ions regenerates the (**6**)/(**7**) duplex, and the catalytic functions of the β-CD are switched off.

Figure 2(B) depicts the switchable catalytic functions of the β-CD units in the scaffold in the presence of K^+/18-crown-6-ether

as triggers. Assembly of the cooperatively K^+-stabilized G-quadruplex duplex scaffold consisting of (**6**)/(**7**) results in a switched off catalytic assembly, where the adamantane moiety blocks the β-CD cavity and inhibits the hydrolysis of (**3**). Addition of 18-crown-6-ether to the system leads to the elimination of K^+ ions from the G-quadruplex, and its separation. The separation of the G-quadruplex yields an unstable duplex that dissociates to the components (**6**) and (**7**). The free β-CD-modified strand catalyzes the hydrolysis of (**3**), Figure 2(B), point (a), resulting in the switched-on catalyst. Re-addition of K^+ ions to the system regenerates the K^+-stabilized G-quadruplex duplex (**6**)/(**7**) where the catalytic hydrolysis of (**3**) is switched off, Figure 2(B), point (b). By subjecting the system alternatively to K^+ ions and 18-crown-6-ether as chemical stimuli, the system can be switched between "OFF" and "ON" catalytic states.

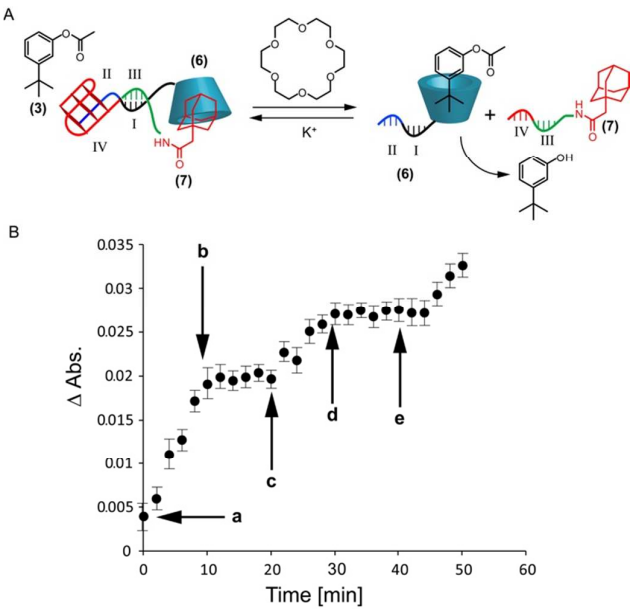

Fig. 2. (A) Switchable "ON"/"OFF" catalyzed hydrolysis of *m-tert*-butylphenyl acetate using the allosterically G-quadruplex stabilized (**6**)/(**7**) system as regulating scaffold. The separation of the (**6**)/(**7**) regulating scaffold by means of 18-crown-6-ether switches on the catalytic process, while the K^+-induced stabilization of the (**6**)/(**7**) complex, switches off the hydrolysis of (**3**). (B) Time depended absorbance changes upon the cyclic "ON" and "OFF" operation of the β-CD catalyzed hydrolysis of (**3**): (a) the experiment is initiated in the presence of (**6**), (**7**), and (**3**). (b) Time indicating addition of K^+ (20 mM) to the system. (c) Time indicating addition of 18-crown-6-ether (20 mM). (d) and (e) indicate time of sequential further additions of K^+ (20 mM) and 18-crown-6-ether (20 mM) respectively. Error bars derived from N= 3 experiments.

The switched "OFF" catalytic system shown in Figure 2(B) includes the K^+-stabilized G-quadruplex as a functional unit to induce the "OFF"-state of catalytic reaction. It is, however, well-established that hemin (**8**) binds to G-quadruplex to reveal catalytic activities mimicking the properties of the native horseradish peroxidase, HRP, hemin/G-quadruplex horseradish peroxidase mimicking DNAzyme.[15] For example, it was reported that in analogy to HRP, the hemin/G-quadruplex catalyzes the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), $ABTS^{2-}$, by H_2O_2 to form the colored product, $ABTS^{\cdot-}$.[16] Similarly, the hemin/G-quadruplex was found to catalyze the generation of chemiluminescence via the H_2O_2-mediated oxidation of luminol.[17] The formation of the cooperatively K^+-stabilized G-quadruplex duplex scaffold (**6**)/(**7**) suggests, however, that the system could be programmed to exhibit double switchable "ON"/"OFF" catalytic functions, as outlined in Figure 3(A).

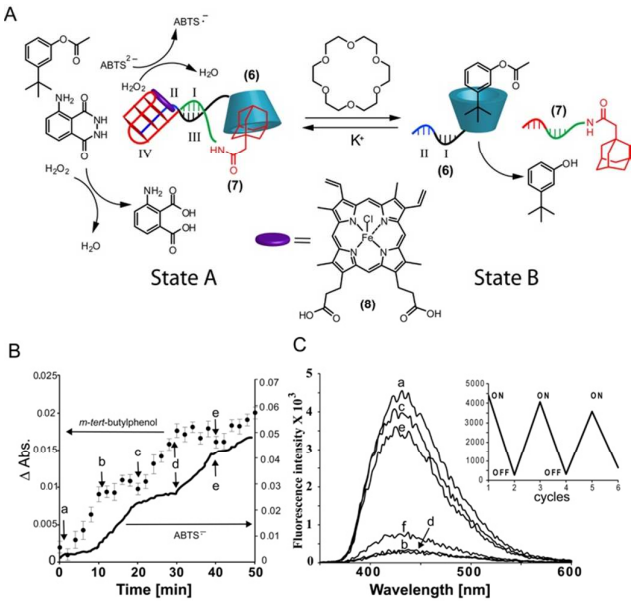

Fig. 3. (A) Dual switchable triggering of two catalytic processes using a hemin/G-quadruplex (DNAzyme) and β-cyclodextrin/adamantane as hybrid regulating scaffold. The association of hemin to the G-quadruplex–stabilized (**6**)/(**7**)-β-cyclodextrin/adamantane scaffold activates the hemin/G-quadruplex horseradish peroxidase mimicking DNAzyme (catalyzed H_2O_2 oxidation of $ABTS^{2-}$ to $ABTS^{\cdot-}$ or catalyzed oxidation of luminol by H_2O_2 to yield chemiluminescence). Under these conditions, the β-cyclodextrin-catalyzed hydrolysis of (**3**) is switched off. Dissociation of the (**6**)/(**7**) scaffold in the presence of 18-crown-6-ether yield the separated oligonucleotide (**6**) where the β-cyclodextrin catalyses the hydrolysis of (**3**) and the DNA functions are switched off. (B) Time dependent absorbance changes observed upon the switchable operation of the DNAzyme catalyzed oxidation of $ABTS^{2-}$ by H_2O_2 or β-cyclodextrin catalyzed hydrolysis of (**3**) using 18-crown-6-ether and K^+ as triggers and the (**6**)/(**7**) regulating system. Right: switchable absorbance changes upon the DNAzyme catalyzed oxidation of $ABTS^{2-}$ to $ABTS^{\cdot-}$ (λ= 415 nm). Left: switchable absorbance changes upon the β-cyclodextrin-catalyzed hydrolysis of (**3**). (a) Time intervals that correspond to the system in state "B", in the presence of 18-crown-ether. The time intervals marked (b) correspond to the system in state "A" where K^+ is added. (C)

Switchable chemiluminescence spectra corresponding to (a), (c), and (e) the system is in state "A". (b), (d), (f) Correspond to the system in state "B". Inset: Switchable chemiluminescence intensities generated by state "A"-switch "ON" and state "B"-switch "OFF", respectively.

The association of hemin, (**8**), to the G-quadruplex-stabilized duplex (**6**)/(**7**) yields the active hemin/G-quadruplex DNAzyme that catalyzes the oxidation of $ABTS^{2-}$ to $ABTS^{\cdot-}$ by H_2O_2 or catalyzes the generation of chemiluminescence via the H_2O_2-mediated oxidation of luminol. Under these conditions where the catalytic functions of the hemin/G-quadruplex are switched on, the catalytic functions of the β-CD units are switched off due to their blockage by the adamantane units. Addition of 18-crown-6-ether to the system induces the separation of G-quadruplex unit and to depletion of the catalytic functions of the hemin/G-quadruplex. The separation of the G-quadruplex leads to the dissociation of the duplex (**6**)/(**7**), resulting in the switched-on catalytic functions of the free β-CD strand (**6**), toward the hydrolysis of (**3**). That is, by subjecting the system consisting of (**6**), (**7**) and hemin to the external chemical stimuli K^+ and 18-crown-6-ether, dual switchable catalytic responsiveness is observed. In the presence of K^+ ions the hemin/G-quadruplex DNAzyme is switched-on and the catalytic functions of the β-CD units are switched off. In turn, in the presence of 18-crown-6-ether the hemin/G-quadruplex dissociates, depleting the catalytic functions of the hemin/G-quadruplex DNAzyme, and the catalytic functions of the separated β-CD strand (**6**) are switched-on. Figure 3(B) shows the dual catalytic functions of the hemin/G-quadruplex/ β-CD scaffold upon the cyclic addition of K^+ ions and 18-crown-6-ether, where the catalytic functions of the hemin/G-quadruplex are followed by the H_2O_2-mediated oxidation of $ABTS^{2-}$, and the catalytic function of the β-CD are probed spectroscopically by following the *m-tert*-butylphenolate product generated upon hydrolysis of (**3**). Figure 3(C) shows the catalytic functions of the system subjected to K^+ ions and 18-crown-6-ether by following the chemiluminescence generated by the hemin/G-quadruplex DNAzyme-stimulated oxidation of luminol by H_2O_2.

The present study has demonstrated the controlled switchable catalytic functions of a molecular receptor (β-CD) by means of an inhibitor unit (adamantane) within reconfigurable DNA scaffolds. One scaffold consisted of a duplex-structure that was reversibly reconfigured by means of fuel/anti-fuel strands. The second system involved a G-quadruplex-stabilized DNA scaffold that was reversibly reconfigured between a G-quadruplex catalytically inactive scaffold, and a random-coil separated system exhibiting catalytic activity using K^+ and 18-crown-6-ether as chemical triggering stimuli. Furthermore, the incorporation of hemin into the G-quadruplex unit resulted in a supramolecular β-CD/DNAzyme scaffold revealing dual switchable catalytic functions in the presence of the K^+ ion/18-crown-6-ether stimuli. In the presence of K^+ ions, the catalytic functions of the hemin/G-quadruplex horseradish peroxidase mimicking DNAzyme were switched-on, whereas the catalytic hydrolytic functions of β-CD are switched off. In turn, in the presence of 18-crown-6-ether, the catalytic functions of the DNAzyme are switched-off, and the hydrolytic functions of the β-CD units are switched-on.

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