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Switchable Supramolecular Catalysis using DNA-Templated Scaffolds

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Instrumentation

Absorbance measurements of $ABTS^{\cdot-}$ were performed using a Shimadzu UV-2401PC UV/Vis spectrophotometer. Mass spectra were obtained using a 6520 Accurate-Mass Q-TOF LC/MS-Agilent Technologies instrument. The NMR spectra were recorded using an Advance DRX 400 Bruker instrument and the spectra were referenced to residual solvent signals and were recorded at 298 K. ^{1}H NMR data are reported as follows: chemical shift in ppm on the δ scale, multiplicity, and integration (s, singlet; d, doublet; m, multiplet). ^{13}C NMR data are reported as follows: chemical shift in ppm on the δ scale.

Materials and general procedures

Unless otherwise noted, all materials and compounds were prepared using commercially available reagents and used without further purification. Water was purified with a Milli-Q purification system. Dibenzocyclooctyne-sulfo-N-hydroxysuccinimidyl ester, 6-monodeoxy-6-monoamino-β-cyclodextrin hydrochloride, 1-adamantaneacetic acid, N-hydroxysulfosuccinimide sodium salt, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide, sodium carbonate, 2-[morpholino]ethanesulfonic acid (MES), $ABTS^{2-}$, sodium chloride, magnesium chloride, hydrogen peroxide, 3-tert-butylphenol, acetyl chloride, luminol, and potassium chloride, were purchased from Sigma–Aldrich. DNA oligonucleotides were purchased from Integrated DNA Technologies Inc., (Coralville, IA). Hemin was purchased from Porphyrin Products (Logan, UT).

Sequences of the oligonucleotides.

(1): 5′-/5AzideN/GGC TAG CGT TAC TCG G-3′

(2): 5′-CGT ATT ATC CGA GTA ACG CTA GCC/3AmMC6T/-3′

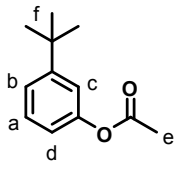
(4): 5′-AGTGGAAAGGCTAGCGTTACTCGGATAATACG-3′

(5): 5′-CGTATTATCCGAGTAACGCTAGCCTTTCCACT -3′

(6): 5′-/5AzideN/ACG TAA TTG GGT -3′

(7): 5′-GGG CGG GAT GGG TAT TAC GT/3AmMC6T/-3′

***m-tert*-Butylphenyl acetate (3) was prepared following published procedures.[1]**

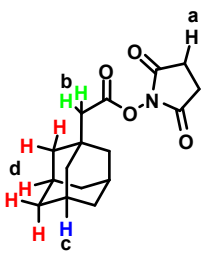


^{1}H NMR: ($CDCl_3$, 400 MHz): 7.27 (m, $1H_a$), 7.27 (m, $1H_b$), 7.10 (s, $1H_c$), 6.94 (d, J = 7.2 Hz, $1H_d$), 2.32 (s, $3H_e$), 1.34 (s, $9H_f$). ES-MS (m/z): 215.10 [$C_{12}H_{16}O_2$ + Na^+] (cal. 215.10).

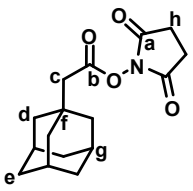
Synthesis of N-hydroxysuccinimide adamantane acetate.

1-Adamantane acetic acid (200 mg, 1 mmol, MW 194.27 $gmol^{-1}$) was dissolved in dry DCM, and cooled down to 0 °C. $SOCl_2$ (184 mg, 1.5 equiv. MW 118.97 $gmol^{-1}$) was added dropwise. After 10 minutes triethyl amine was added to the reaction mixture and the resulting solution was let to react for 3 hours at room temperature. After this period of time NHS (178 mg, 1.5 equiv.

MW 115.09 $gmol^{-1}$) was added to the reaction flask and let to react for 12 hours. Then the solvent was removed under reduced pressure and the product was washed with aqueous HCl (0.1 M), the organic phase was extracted in DCM and subjected to column chromatography (r.f.= 0.15 hexane:ethyl acetate, 9:1). 74% yield.



^{1}H NMR ($CDCl_3$, 400 MHz): 2.85 (m, $4H_a$), 2.36 (s, $2H_b$), 2.03 (s, 3 H_c), 1.71 (m, 12 H_d), ES-MS (m/z): 314.14 [$C_{16}H_{21}NO_4$+ Na^+] (cal. 314.14).



^{13}C NMR ($CDCl_3$, 125 MHZ): (a) 169.2, (b) 166.3, (c) 45.3, (d) 42.0, (e) 36.5, (f) 33.2, (g) 28.5, (h) 25.7.

Syntheses of β-CD-modified and adamantane-modified oligonucleotides

Dibenzocyclooctyne-sulfo-N-hydroxysuccinimidyl ester (1 mg, 1.88 µmol, MW 532.50 $gmol^{1-}$) was mixed with 1.2 equiv. of 6-monodeoxy-6-monoamino-β-cyclodextrin hydrochloride (2.64 mg, 2.25 µmol, MW 1170.46 $gmol^{1-}$) in PBS buffer (10 mM, pH 7.5) for one hour. Following the reaction time, the mixture was added to the azide-modified oligonucleotides (**1)** or (**6)**. The resulting solution was allowed to react for 2 hours at room temperature. The product was cleaned using a MicroSpin G-25 columns and further purified using reverse-phase HPLC. (**1**) ES-MS (m/z): found 6680.01, (cal. 6680.9) (**6**) ES-MS (m/z): found 5444.2, (cal. 5467.3)

150 µL of a stock solution of amino-modified oligonucleotide (**2**) or (**7**) (200 µM in H_2O) was mixed with 100 µL of phosphate buffer (100 mM pH 7.2) and 20 µL of dimethylformamide. To

the resulting solution, 40 μL of a stock solution of N-hydroxysuccinimide adamantane acetate. in dimethylformamide was added in fractions of 10 μL over a period of 4 hours with continuous vigorous shaking. The mixture was let to react overnight and product was cleaned using a MicroSpin G-25 columns and further purified using reverse-phase HPLC. (**2**) ES-MS (m/z): found 7956.3, (cal. 7948.9) (**7**) ES-MS (m/z): found 6904.1, (cal. 6905.2)

β-Cyclodextrin-oligonucleotide catalyzed hydrolysis of *m-tert*-butylphenyl acetate (3).

Switchable catalysis of (**3**) was studied in a solution consisting of (**1**) (10 μM), (**2**) (10 μM), and (**3**) (100 μM) in HCO_3^-/CO_3^{2-} (10 μM pH 9.2) and $MgCl_2$ (5 mM) at 30 °C. Fuel (**4**) and anti-fuel (**5**) oligonucleotides were added at 10 mM. The reaction was followed by measuring the absorbance of the product *m-tert*-butylphenol (λ= 275 nm).

The switchable catalysis of **3** (100 μM) by the K^+-stabilized system was studied in a solution consisting of **6** (10 μM), **7** (10 μM), in HCO_3^-/CO_3^{2-} (10 mM pH 9.2) and $MgCl_2$ (5 mM) at 30 °C. Alternated additions of K^+ (20 mM) and 18-crown-6-ether (25 mM) were used to switch the system "OFF" and "ON" respectively.

The switchable HRP-mimicking activities of the hemin–G-quadruplex system were analyzed using hemin and $ABTS^{2-}$ with final concentrations of 100 nM and 2 mM, respectively. The reaction was initialized by addition of hydrogen peroxide (200 mM). The "OFF"/"ON" switching was generated by the addition of K^+ (20 mM) or 18-crown-6 (25 mM), respectively. The rate of the peroxidase-mimicking reaction was monitored at λ= 415 nm.

The chemiluminescence assays were performed in a solution that included **6** (10 μM) and **7** (10 μM), 100 nM of hemin, 0.5 mM of luminol, and 20 mM of H_2O_2 in HCO_3^{1-}/CO_3^{2-} (10 mM pH 9.2) and $MgCl_2$ (5 mM). The light emission intensity was measured immediately after the

addition of H_2O_2. The "OFF"/"ON" switching was generated by the addition of K^+ (20 mM) or 18-crown-6 (25 mM), respectively.

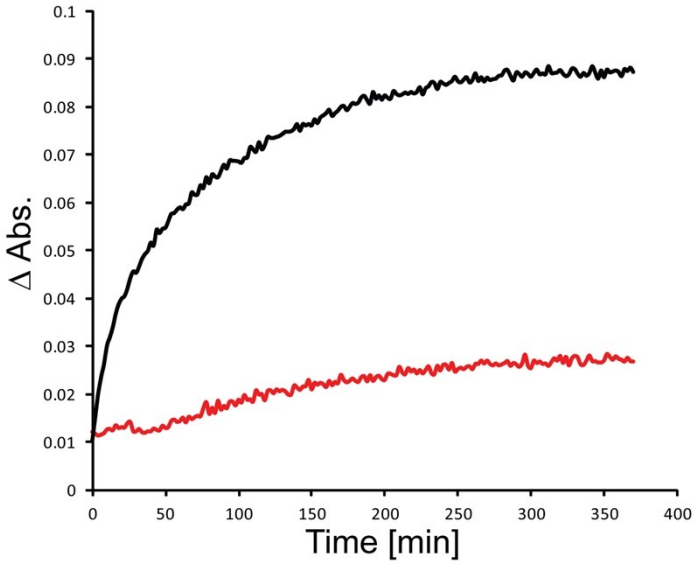


Figure S1. Time dependent change in absortion upon hydrolysis of *m-tert*-butylpheynyl acetate (**3**). Black line: using β-cyclodextrin functionalized oligonucleotide (**1**) (10 μM). Red line: Background hydrolysis of *m-tert*-butylpheynyl acetate (**3**) 100 μM, HCO_3^-/CO_3^{2-} ,10 mM, pH= 9.2).

References

1.- (a) van Etten, R. L.; Sebastian. J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc*. **1967**, 89,3242-3253,3253-3262.