DNA Switches

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A Robust Electronic Switch Made of Immobilized Duplex/Quadruplex DNA**

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In recent years, DNA and RNA have been used extensively as materials for the construction and self-assembly of a variety of nanoscale devices.[1–3] DNA-based mechanical switches have been described recently.[1] Extension–contraction transitions of DNA nanoconstructs (e.g., G-quadruplexes) upon ligand binding have also been reported.[2–3] A G-quadruplex (G4-DNA) is a quadruple-helical DNA secondary structure, in which guanines are paired by Hoogsteen hydrogen bonds to form guanine base quartets.[4] G4-DNA is stabilized by the coordination of specific metal ions, such as K^+ ions or Sr^{2+} ions, which bind between successive G-quartets.[5] G4-DNA-based nanoconstructs have been used as sensors to detect the blood protein thrombin,[6–8] K^+ ions,[8–14] and target oligonucleotides[9,15] by binding of these analytes to DNA aptamers (nucleic acid receptors that are themselves sometimes G4-DNA[7]). The ligand binding properties of G4-DNA, as well as its ability to conduct electrical charges, have been characterized extensively by using gel electrophoresis and spectroscopic methods.[6–17]

G4-DNAs typically form by the folding of G-rich single-stranded DNA (ssDNA), but such ssDNA to G4-DNA transitions are not easily reversed. In contrast, the duplex DNA (dsDNA) to G4-DNA transition can be kinetically more favored, brought about by the addition and removal of K^+ ions (utilizing a chelator such as [18]crown-6). We recently reported a biochemical study of the charge-conducting properties of a contractile DNA nanoswitch, which is able to switch repeatedly between a structurally extended electronic "off" state (an extended duplex) and a contracted electronic "on" state (a G-quadruplex).[3] The conductivity of the K^+-induced contracted duplex in solution was much higher than that of the extended conformational state. This biochemical study of conductivity,[3] however, provided no direct indication of whether such a device could be adapted for practical use (i.e., whether electronic switching would still be observed if the device were to be localized on an electronic chip).

Herein we report the results of our first chip-based strategy, which is able to directly and efficiently measure both the reversibility and the repeatability of the electronic switching behavior of the dsDNA/G4-DNA constructs. Specifically, we present an electrochemical (square-wave voltammetry) investigation of the DNA nanoswitch immobilized on a gold electrode.

As shown in Figure 1, the contractile DNA duplex incorporates two short, separated motifs of G/G mismatches that are flanked by Watson–Crick base-paired DNA. The duplex is labeled with a ferrocene (Fc) moiety (an electroactive species) at the 5′ terminus of one strand. The 5′ end of the other DNA strand is attached to a gold electrode by Au–S bond formation. The DNA-modified electrode is then treated with 6-mercaptohexanol to minimize any nonspecific adsorption of DNA to the electrode.

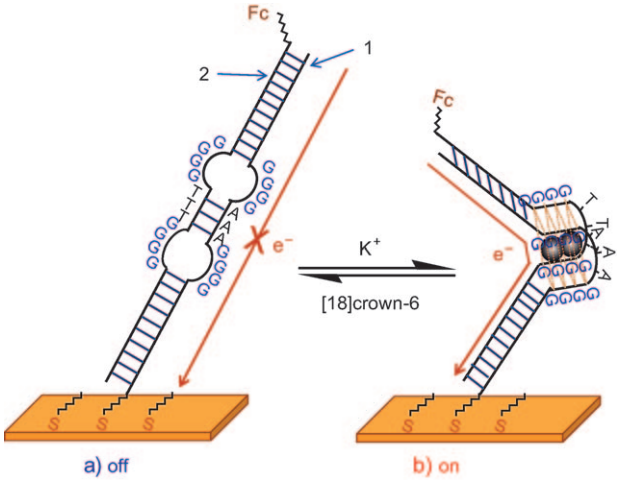

Figure 1. DNA nanoswitch immobilized on a gold electrode. a) "Off" state; b) "on" state induced by the addition of KCl. **1**: HS-$(CH_2)_6$-O-5′-TTT AGC TCG AGA GGG GAA AGG GGA GAC GCT GGA GA-3′; **2**: Fc-$(CH_2)_6$-O-5′-TC TCC AGC GTC TGG GGT TTG GGG TCT CGA GCT AAA-3′.

Figure 2a shows square-wave voltammograms of the surface-immobilized DNA contractile duplex in 50 mM LiCl and 10 mM tris(hydroxymethyl)aminomethane (Tris) buffer, at pH 7.4. In the absence of K^+ ions, the oxidation (Fc to Fc^+) current is as low as 20 nA (dotted line in Figure 2a). Incubation of the electrode in 10 mM potassium chloride for 1 h led to an apparent increase of the current up to 160 nA

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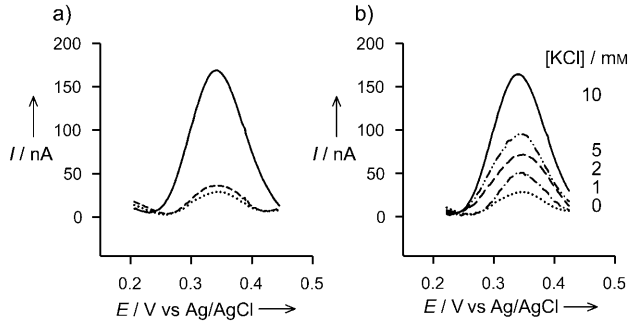

Figure 2. Square-wave voltammograms of the DNA switch in 50 mM LiCl and 10 mM Tris at pH 7.4 (background was subtracted) a) in the absence (dotted line) and in the presence (solid line) of 10 mM KCl and after further treatment with 1 mM [18]crown-6 (dashed line); b) in the presence of different concentrations of KCl.

(solid line in Figure 2a); a subsequent treatment with [18]crown-6 (1 mM) resulted in a decrease of the signal to the originally observed low level (dashed line in Figure 2a). It was also confirmed that the electrochemical signal of the surface-immobilized DNA switch depends on the K^+ ion concentration (up to 10 mM, Figure 2b). For a better understanding, the increase in oxidation current is plotted as a function of the K^+ ion concentration in Figure 3; on average

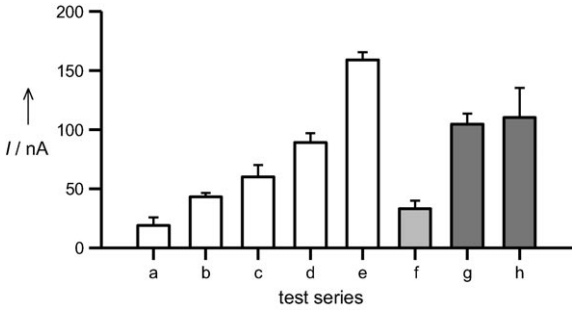

Figure 3. Dependence of the electrochemical signal of the contractile DNA switch on the KCl concentration (in 50 mM LiCl and 10 mM Tris at pH 7.4), with a) 0 mM, b) 1 mM, c) 2 mM KCl, d) 5 mM, e) 10 mM KCl, and f) after further treatment with 1 mM [18]crown-6. Watson–Crick dsDNA g) before and h) after treatment with 10 mM KCl is presented for comparison.

the current changes from (20 ± 7) nA to (160 ± 14) nA, that is, an eightfold increase of the current in the presence of 10 mM K^+ ions is observed. Figure 3 also shows that in the absence of K^+ ions, the oxidation current through the contractile DNA switch is lower than that through a completely Watson–Crick base-paired dsDNA control (which contains no G/G mismatches, but the sequence of which is otherwise identical to that of the contractile DNA switch). In contrast to the contractile switch, no significant current change is observed for the dsDNA control after incubation with potassium chloride (Figure 3g,h). This result indicates that potassium chloride itself has no direct effect on the conduction current through DNA.

We hypothesize that in the absence of K^+ ions, charge conduction through the contractile DNA switch from its 5′-tethered ferrocene to the alkanethiol-passivated gold electrode is interrupted by the G/G mismatches and the intervening three consecutive A–T base pairs, thus resulting in a very low overall electrochemical signal. Such a hypothesis concurs with the earlier, biochemical results.[3] After treatment with K^+ ions, the G/G motifs assemble to form the more conductive intra-duplex G-quadruplex (Figure 1b), in turn promoting charge transfer and giving rise to a much higher electrochemical signal. The subsequent addition of a potassium chelator ([18]crown-6) reverses the contraction induced by K^+ ions and restores the signal to its original low level.

The switching between the extended and the contracted forms of the contractile DNA duplex has been demonstrated biochemically,[3] yet the repeatable reversibility of such a DNA-based nanoswitch can be measured only indirectly by using the gel electrophoresis method. The present system uses surface-immobilized contractile DNA duplexes for direct electrochemical measurements, therefore establishing a viable protocol for real-time monitoring of the reversibility and repeatability of the on–off transition of the contractile DNA nanoswitch. As shown in Figure 4, repeated addition

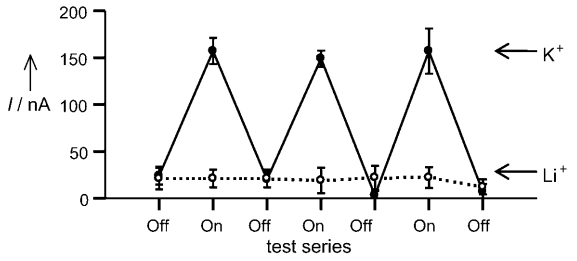

Figure 4. Repeated switching between on and off states of the contractile DNA switch, induced by KCl. Solid line (K^+ ions): Incubation in 10 mM KCl/buffered 50 mM LiCl for 1 h (on); followed by treatment with 20 mM [18]crown-6 for 1 h (off). Dashed line (Li^+ ions): control, incubated as above.

and removal of the effector (K^+ ions) gives rise to a highly robust and reversible on–off switching of the conduction current, while addition of Li^+ ions does not affect the signal at all. This observation provides further evidence that the reversible structural transitions between the extended and the contracted forms of the contractile duplex dictates the state of the electronic switch, with the contracted conformation defining the on state and the extended conformation defining the off state (Figure 1). Reversible extension and contraction of the contractile DNA nanoswitch can therefore be repeatedly realized at a solid–liquid interface, and can be monitored directly by using electronic measurements.

The chip-based results are consistent with the earlier biochemical data in proposing charge conduction through the DNA nanoswitch, although we cannot formally rule out the effect of a change in distance between the redox-active species and the gold electrode. The formation of G4-DNA in the contracted mode of the nanoswitch may bring the ferrocene closer to the gold surface and therefore enhance

the current signal. Such proximity effects induced by conformational changes (from ssDNA to G4-DNA) have been reported previously.[18] Regardless of this possibility, the key observation in the present report is that the measured electronic switching between the on and off states of the contractile nanoswitch directly parallels its conformation-switching behavior (together with the predicted modulation of its conductivities).

In summary, we have demonstrated that a practical nanoelectronic switch can be designed and utilized on a conductive electronic chip by using contractile DNA duplexes as the switchable medium. Such a prototype should find real-time application in both the design of DNA-based nanocircuitry as well as in sensing applications for various analytes.

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