DNA Nanotechnology

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Ion-Selective Formation of a Guanine Quadruplex on DNA Origami Structures**

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Abstract: *DNA origami nanostructures are a versatile tool that can be used to arrange functionalities with high local control to study molecular processes at a single-molecule level. Here, we demonstrate that DNA origami substrates can be used to suppress the formation of specific guanine (G) quadruplex structures from telomeric DNA. The folding of telomeres into G-quadruplex structures in the presence of monovalent cations (e.g. Na^+ and K^+) is currently used for the detection of K^+ ions, however, with insufficient selectivity towards Na^+. By means of FRET between two suitable dyes attached to the 3′- and 5′-ends of telomeric DNA we demonstrate that the formation of G-quadruplexes on DNA origami templates in the presence of sodium ions is suppressed due to steric hindrance. Hence, telomeric DNA attached to DNA origami structures represents a highly sensitive and selective detection tool for potassium ions even in the presence of high concentrations of sodium ions.*

DNA can be folded into almost any 2D and 3D shape by using the DNA origami technique.[1] DNA nanostructures can be decorated with proteins, nanoparticles, fluorescent dyes, and higher-order DNA structures with nanometer precision, and thus they can serve as a versatile tool in analytical science.[2] DNA origami structures can be used as a platform to detect RNA sequences[3] and other molecular species through atomic force microscopy,[4] for few-molecule detection using surface-enhanced Raman scattering (SERS),[5] and to determine the yield of sequence-specific DNA damage.[6] The formation of guanine (G) quadruplex structures from telomeric DNA sequences was also extensively studied using a DNA origami frame and high-speed AFM.[7] Telomeres are located at the ends of eukaryotic chromosomes, and stabilize and protect the genome.[8] The G-rich, single-stranded mammalian telomeres have the sequence 5′-$(TTAGGG)_n$[9] and they can form nonduplex structures in the presence of monovalent cations. In the nonduplex structure four G bases are associated with eight stable hydrogen bonds to form a G-tetrad. Two or more G-tetrads are stacked to form a G-quadruplex. Since the association constant for G-quadruplex formation is lower for Na^+ than for K^+ ions,[10,11] telomere sequences have been suggested as selective K^+ sensors using Förster resonance energy transfer (FRET).[10–12] However, Na^+ induces G-quadruplex formation from free human telomeric DNA in a concentration range from 10–205 mM. Under physiological conditions (ca. 145 mM Na^+) this can lead to considerable errors in the determination of K^+ concentration. As we demonstrate here, by using telomeric DNA attached to DNA origami platforms, the formation of G-quadruplexes by Na^+ is completely suppressed, whereas the K^+-induced G-quadruplex formation is not influenced.

In the present study, we have used FRET to investigate in detail the K^+- and Na^+-induced folding of G-quadruplexes from both free telomere sequences and telomere sequences attached to triangular DNA origami platforms. FRET refers to a nonradiative energy transfer from an excited donor to an acceptor through dipole–dipole interactions. The FRET efficiency η is strongly distance-dependent according to Equation (1).

$$\eta = \frac{R_0^6}{R_0^6 + R^6} \tag{1}$$

Here, R is the donor–acceptor distance and R_0 the Förster radius at which the FRET efficiency is 50%. The FRET efficiency can also be calculated based on the donor's decay time [Eq. (2)].

$$\eta = 1 - \frac{\tau_{DA}}{\tau_D} \tag{2}$$

Here, τ_D is the donor's decay time in the absence of the acceptor and τ_{DA} is the decay time in the presence of the acceptor.[13]

In the present study, free human telomeric DNA was used as a reference system modified with cyanine 3 (Cy3) at the 5′-end and fluorescein (FAM) at the 3′-end (5′-Cy3-TTG GGA TTG GGA TTG GGA TTG GGA TT-FAM). When K^+ or Na^+ is added to the solution, the conformation changes from

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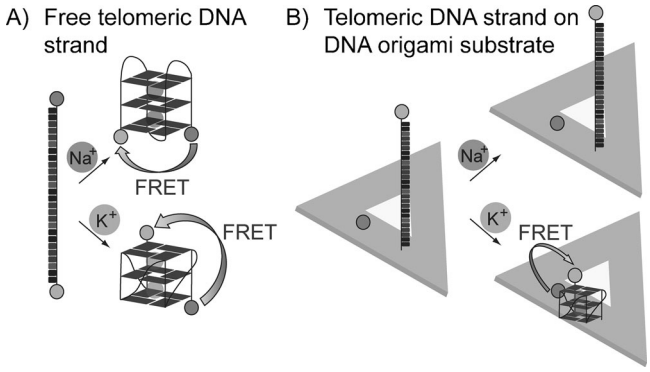

Figure 1. Scheme of the telomere-based potassium-sensing systems. A) Free telomeric DNA strand (5′-Cy3-TT(GGGA)$_4$TT-FAM, T = black, G = dark gray, A = light grey) with fluorescein (FAM, dark gray) as the donor dye and cyanine3 (Cy3, light gray) as the acceptor dye. The DNA strand folds into G-quadruplex structures when monovalent cations such as K^+ and Na^+ are present. The different cations induce the formation of different G-quadruplex structures. When the free telomeric DNA strands are used, both G-quadruplexes can be formed and K^+ and Na^+ are detected using FRET. B) The telomeric DNA strand is immobilized on the DNA origami structure as a protruding strand attached to one staple strand. The telomeric DNA strand is placed 3.3 nm away from FAM on the DNA origami structure. The G-quadruplex can only be folded in the presence of potassium ions.

a single-stranded random coil to a compact G-quadruplex structure, in which the donor–acceptor distance is 4.6 nm in the presence of K^+ and 5.8 nm in the presence of Na^+ (see Figure 1 A and Figure S1 in the Supporting Information (SI) for details). In the second system, in which triangular DNA origami templates are used (AFM image is shown in Figure S2), FAM was placed 3.3 nm away from the 3′-end of the protruding telomeric DNA. The latter is modified with Cy3 at its 5′-end (Figure 1 B and Figure S1). In the compact G-quadruplex structure the donor–acceptor distance has a maximum value of 7.5 nm.

Steady-state emission spectra were recorded at a DNA concentration of 5 nM with an excitation wavelength of 450 nm and are shown in Figure 2. Figure 2 A and B show the fluorescence emission spectra for the free telomeric DNA sequence upon addition of KCl (A) and NaCl (B). In Figure 2 C and D the respective spectra are shown for the telomeric DNA attached to DNA origami triangles. When no FRET occurs, the emission of FAM at 515 nm is dominant. Since in the random-coil conformation the organic dyes are already close enough for FRET to occur, weak Cy3 emission at 565 nm can be observed in the steady-state spectra (Figure 2 A,B) also when no salt is present. When either KCl or NaCl is added to the free telomeric DNA (Figure 2 A,B), the dyes are brought closer together due to G-quadruplex formation and more efficient FRET can be observed. FAM fluorescence is quenched and simultaneously Cy3 emission increases. The addition of 1 mM KCl (Figure 2 A) is sufficient to change the emission properties. As the association constant for Na^+ [11] is lower than that for K^+, a clear change in the fluorescence emission is observed upon addition of about 25 mM NaCl (Figure 2 B). However, this distinction is insufficient under biologically relevant conditions, since in extracellular liquids the Na^+ concentration is roughly 145 mM and the K^+ concentration is as low as 4 mM. As can be seen in Figure 2 B the single telomeric DNA strand shows high sensitivity for Na^+ in a concentration range of 25–205 mM. Thus, the use of free telomeric DNA as a selective potassium sensor in biological media is not feasible. In order to overcome this problem, the telomeric DNA was immobilized on DNA origami structures to construct the DNA origami/telomere FRET sensing system. Figure 2 C shows the response of the DNA origami/telomere system to increasing amounts of KCl. Overall, the FRET efficiencies are lower since the donor–acceptor distance in the DNA origami design is larger than that in the free telomeric system (Figure 1). Nevertheless, a clear change both in the donor and acceptor emission is observed in the concentration range between 5 mM and 110 mM KCl. The Cy3 emission intensity decreases slightly at 0.1–2.5 mM KCl owing to moderate heating to 40 °C with the first KCl addition, which might increase the average distance between the dyes in the nonfolded telomeres. Starting at a concentration of 5 mM KCl the FRET efficiency increases clearly. The situation is completely different when NaCl is added to the DNA origami/telomere structures (Figure 2 D). The steady-state fluorescence emission spectra are unaffected by Na^+ ions even at concentrations as high as 205 mM. That is, the average donor–acceptor distance does not change with increasing NaCl concentration, indicating that the G-quadruplex formation does not take place on DNA origami structures in the presence of Na^+ ions. In Figure 2 E it is demonstrated that the high sensitivity for K^+ is preserved even in the presence of 145 mM NaCl. It should be noted that FRET is also not observed upon addition of NaCl to the DNA origami/telomere system when one of the dyes is placed directly beside the 3′-end of the telomere sequence to mimic the free-telomeric DNA FRET system (see Figure S3 (SI)).

Based on the fluorescence intensity of FAM and Cy3, the association constants are calculated for the free telomeric DNA system to be $1.5 \times 10^4\,M^{-2}$ for K^+ and $33.7\,M^{-2}$ for Na^+ (see Figure S4 (SI)). The association constant for the DNA origami/telomere system is determined to be $2.6 \times 10^4\,M^{-2}$ for K^+ (Figure S4 (SI)), which is very close to the value obtained for the free telomeric DNA with KCl addition. In the presence of 145 mM NaCl, an association constant of $1.4 \times 10^4\,M^{-2}$ in the presence of KCl was obtained (Figure S4 (SI)). This indicates that the formation of the G-quadruplex, and therefore, the sensitivity towards K^+ is virtually unaffected by the DNA origami platform and the presence of Na^+.

Since the steady-state measurements depend on the absolute DNA concentration, the FRET efficiencies have also been determined using time-resolved fluorescence spectroscopy. The fluorescence decay times determined for the donor are summarized in Table 1 (decay curves are shown in Figure S5), and the FRET efficiency is determined using Equation (2). In Figure 3 the FRET efficiencies are plotted *versus* the different salt concentrations.

For free telomeric DNA, the donor decay time decreases with the increase in KCl and NaCl concentration. Accordingly, the FRET efficiency increases with an increase in KCl and NaCl concentration. The dynamic range in the FRET efficiency for K^+ is from about 0.5 mM to 50 mM. On the other

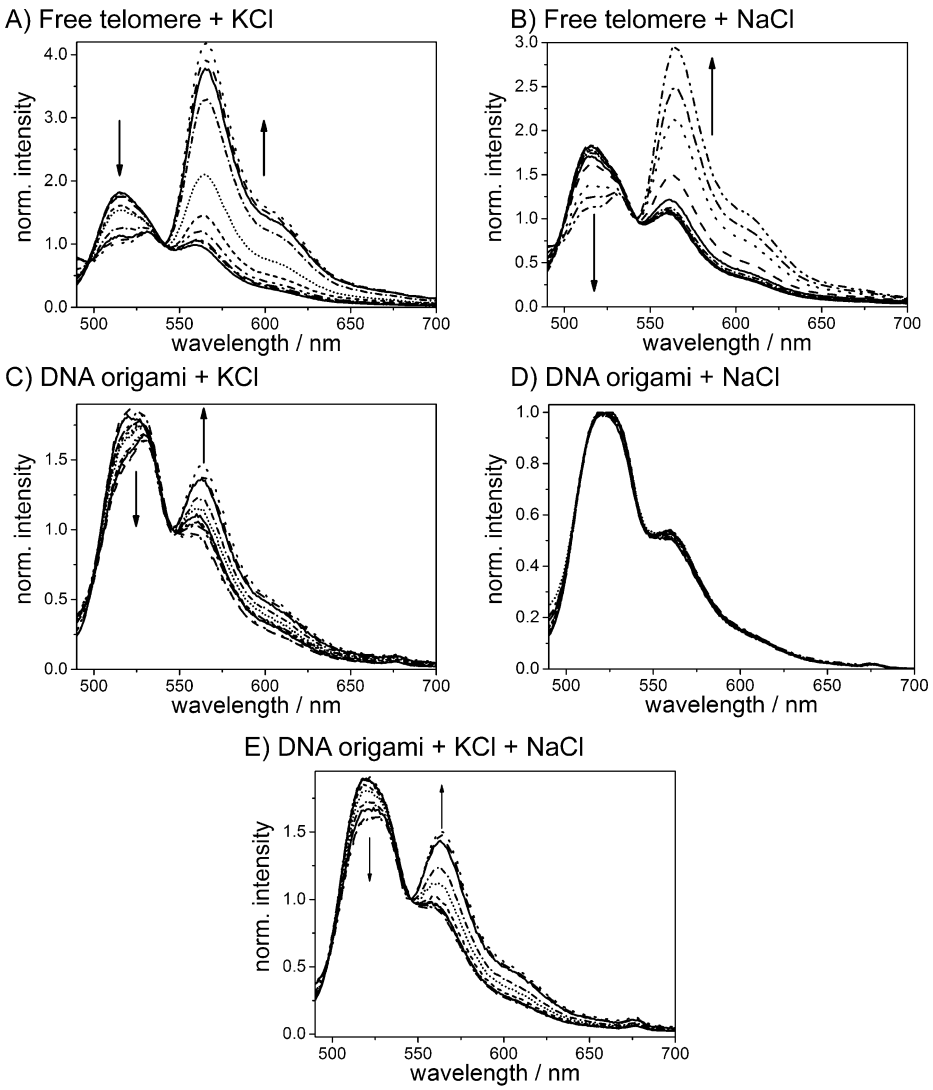

Figure 2. Normalized emission spectra (λ_{ex} = 450 nm) of free telomeric DNA (c = 5 nM) (A,B) and telomeric DNA on DNA origami structures (C,D,E) for various salt concentrations (KCl and NaCl). A,B) Addition of KCl (A; c = 0–110 mM) and NaCl (B; c = 0–205 mM) results in a decrease of the emission intensity of the donor dye FAM (515 nm) and an increase of the emission intensity of the acceptor dye Cy3 (565 nm) with increasing salt concentration due to FRET. Thus, free telomeric DNA is folded into G-quadruplex structures in the presence of both potassium and sodium ions. C,D,E) With the telomeric DNA on DNA origami structures, only KCl addition (C; c = 0–110 mM) leads to a change of the fluorescence emission due to FRET, even in presence of 145 mM NaCl (E; c = 0–110 mM). When NaCl is added (D; c = 0–205 mM), the G-quadruplex structure is not formed on DNA origami substrates and the fluorescence emission of FAM and Cy3 does not change.

hand, for Na^+ the FRET efficiency changes strongly within a wide concentration range from 5 mM to more than 165 mM.

Similar to the case for free telomeric DNA, the FRET efficiency also increases for the DNA origami/telomere system upon the addition of KCl. The differences between the FRET efficiencies of the free telomeric DNA and the DNA origami system (free telomeric DNA: 0.56–0.91; DNA origami substrate: 0.28–0.57) are due to the different donor acceptor–distances in the two systems (Figure 1). When NaCl is added, the FRET efficiency remains unaffected (Figure 3) indicating again that G-quadruplex formation in the presence of Na^+ is suppressed on the DNA origami structures. Consequently, K^+ sensing is also operative at a concentration of 145 mM NaCl (Figure 3).

The formation of G-quadruplexes in the presence of K^+ and the suppression of its formation in the presence of Na^+ with the DNA origami/telomere system can be explained by the specific G-quadruplex structures. Apart from the telomere sequence and the strand polarization, the G-quadruplex structure depends critically on the central metal ion.[14] According to X-ray crystallographic and NMR analysis, Na^+ ions induce a "basket-type" G-quadruplex, whereas in the presence of K^+, a "propeller-type" structure is formed (Figure 1 A).[15,16] The basket-type G-quadruplex has a diagonal loop at the end and therefore its formation is sterically hindered by the DNA origami surface.[15] In contrast, the propeller-type G-quadruplex can still be formed on DNA origami platforms in the presence of K^+ . The formation of the different G-quadruplex structures in the presence of K^+ and Na^+ ions is confirmed by the slightly different donor–acceptor dye distances, which can be determined from the FRET efficiencies for the free telomeric DNA system. The donor–acceptor distances are determined using Equation (1) and the Förster radius R_0 for the FAM–Cy3 FRET pair. R_0 has been calculated to be 6.7 nm based on the spectral overlap (see Figure S6 (SI)). Accordingly, the donor–acceptor distance in the free telomere sequence without the addition of NaCl or KCl is 6.4 nm. Upon the addition of 165 mM KCl the donor–acceptor distance decreases to 4.6 nm indicating the folding of the G-quadruplex. This agrees very well with the expected distance (4.6 nm, Figure S1) based on the X-ray crystallography data[16] and taking into account the length of the linker between the organic dyes and DNA strand (0.7 nm). For 165 mM NaCl the donor–acceptor distance determined from the FRET efficiency is 5.1 nm, which is shorter than the expected distance of 5.8 nm (ΔR(NaCl) = 0.7 nm, Figure S1 (SI)). Here, it should be noted that in the G-quadruplex structures induced by Na^+ (Figure 1 A) the organic dyes are located at the same end of the G-quadruplex. Due to the linker between the organic dyes and the DNA

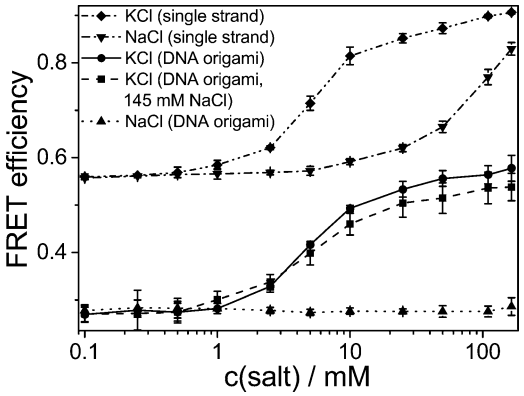

Figure 3. FRET efficiencies based on FAM decay times are plotted versus salt concentrations (KCl (diamonds, circles, squares) and NaCl (triangles pointed down, triangles pointed up)) for the free telomeric DNA ($c = 5$ nM) (diamonds, triangles pointed down) and for telomeric DNA immobilized on DNA origami templates (circles, squares, and triangles pointed up). The FRET efficiency increases with increasing KCl concentration for both systems, even in the presence of 145 mM NaCl (squares). With increasing NaCl concentration only the FRET efficiency of the free telomeric DNA increases. When the telomeric DNA strand is attached to the DNA origami substrate, NaCl is not detectable.

Table 1: Overview of FAM decay times τ for the different systems studied in this work.[a]

c(salt) [mM]	τ (ssDNA, KCl) [ns]	τ (ssDNA, NaCl) [ns]	τ (DNA origami, KCl) [ns]	τ (DNA origami, NaCl) [ns]	τ (DNA origami, KCl in 145 mM NaCl) [ns]
0	1.87	1.89	3.56	3.47	3.47
0.1	1.89	1.90	3.55	3.47	3.50
0.25	1.88	1.89	3.58	3.44	3.50
0.5	1.85	1.87	3.54	3.44	3.48
1	1.79	1.87	3.50	3.45	3.35
2.5	1.63	1.85	3.31	3.47	3.18
5	1.23	1.84	2.96	4.49	2.89
10	0.80	1.76	2.66	3.47	2.59
25	0.64	1.63	2.50	3.48	2.38
50	0.55	1.44	2.40	3.48	2.33
110	0.44	0.99	2.36	3.48	2.23
165	0.40	0.73	2.30	3.43	2.22

[a] Due to FRET the decay time decreases for the free telomeric DNA (ssDNA) after addition of KCl and NaCl, and for the telomeric DNA immobilized on DNA origami substrate (DNA origami) upon addition of KCl and even in presence of 145 mM NaCl. For telomeric DNA on DNA origami structures the decay time is not influenced by the presence of NaCl. τ_D(ssDNA, FAM) = 4.3 ns; τ_D(DNA origami, FAM) = 4.8 ns.

strand, the average distance between FAM and Cy3 might be shorter than expected based on only the G-quadruplex structure.

For the DNA origami/telomere system the donor–acceptor distance decreases from 7.8 nm without added salt to 6.4 nm upon addition of 165 mM KCl due to the formation of G-quadruplexes. The expected maximum distance in this G-quadruplex system in the presence of KCl is 7.4 nm (Figure S1 (SI)). Here it is assumed that the rigid G-quadruplex structure is rotated such that the donor–acceptor distance has a maximum value. The value determined here is an average of all possible rotational conformers and thus is clearly lower than the maximum distance of 7.4 nm.

The sensing system presented in this work is based on "FAM-to-Cy3" FRET on DNA origami substrates and enables selective potassium sensing at concentrations from about 0.5 mM to 50 mM even in the presence of high sodium concentrations (145 mM). To demonstrate this selectivity, we compare the G-quadruplex folding of free telomeric DNA with that of telomeric DNA attached to DNA origami templates. The free telomeric DNA strand shows sensitivity towards both NaCl and KCl, but with different association constants. When telomeric DNA attached to DNA origami structures is used, high selectivity for potassium ions is achieved and the sensitivity of the free telomeric DNA strand is maintained. G-quadruplex formation is completely suppressed on DNA origami structures in the presence of sodium ions due to steric hindrance arising from the the DNA origami surface.

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