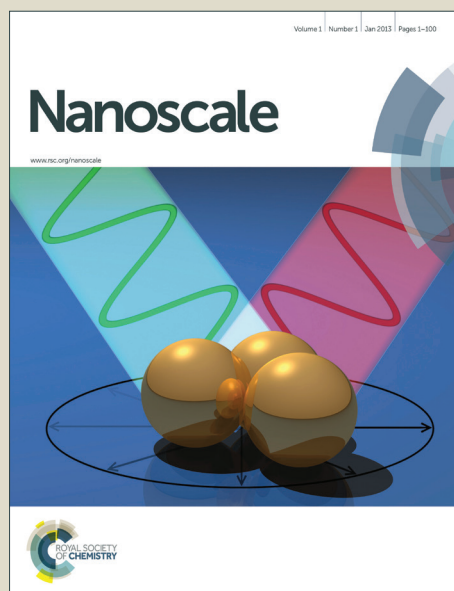


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ARTICLE TYPE

Study of pH-dependence Shrink and Stretch of Tetrahedral DNA Nanostructure

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We monitored the shrink and stretch of the tetrahedral DNA nanostructure (TDN) and the i-motif connected TDN structure at pH8.5 and pH4.5, and we found that not only the i-motif can change its structure when pH changes, but also the TDN and the DNA double helix change their structures when pH changes.

The three dimensional (3D) nucleic acid-based structures have exhibited properties that are often quite different from their linear forms,^[1-15] and it can be used in various research areas. As a potential candidate material of the drug delivery system, the structure changes of the 3D DNA near the solid liquid interface is one of the most important and fundamental aspects of nanoscience.^[16-20]

Tetrahedral DNA nanostructure (TDN) has attracted great interest due to their synthetic simplicity, mechanical rigidity, structural stability, and modification versatility.^[8, 14, 20-22] We designed a DNA tetrahedral structure with i-motif at one vertex and three thiol groups at the other three vertices (Fig.1a, Table S1). This 3D nanostructure is expected to be readily and strongly anchored at Au surfaces by the three thiols at the base of the “pyramid”, leaving i-motif structure at the top. I-motif structure is a type of the relatively DNA structure that could reversibly switch its conformation between a quadruplex, called i-motif (closed state), and a double-stranded (open state) structure depends on the pH.^[23-27]

In this study, we monitored the pH-dependence stretch and shrink of the DNA double helix, TDN and TDN i-motif structure near the solid liquid interface, and we found that not only the i-motif can change its structure when pH changes, but also the TDN and DNA double helix change their structures when pH changes.

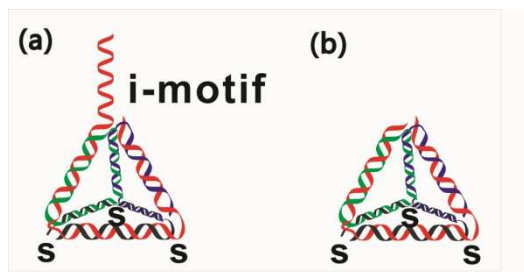


Fig.1./Scheme of tetrahedral DNA nanostructures (a) Tetrahedral DNA nanostructure with i-motif at one vertex and three thiol groups at

the other three vertices (TDN i-motif); (b) Tetrahedral DNA nanostructure (TDN) with three thiol groups.

Polyacrylamide gel electrophoresis had demonstrated that the TDN and TDN i-motif moved more slowly than the ssDNA, confirming the successful assembly of the nanostructure (Fig.2 (a)). The inverted fluorescence microscope images showed that the well assembled TDN was modified on the gold surface (Fig.2 (c)). Even after washing 10 times, the TDN was still strongly adsorbed on the gold surface, and the TDN which was not well assembled (Fig.2 (b)) showed no fluorescence on the surface.



Fig.2./ (a) Polyacrylamide gel electrophoresis of DNA ladder (Line 1), single strand TDN-S5 (Line 2), TDN-S1 (Line 3, with i-motif structure), TDN (Line 4) and TDN with i-motif (Line 5); (b, c) The inverted fluorescence microscope images of (b) not well assembled TDN adsorbed on the gold surface after washing 10 times; (c) well assembled TDN adsorbed on the gold surface after washing 10 times.

To probe the properties of the DNA nanostructures near solid liquid interface, QCM (quartz crystal microbalance) technique was applied. It is very sensitive in detecting small changes in the mass deposition on the surface and the structure or mechanical properties changes of large molecular. QCM is a surface-sensitive technique that relies on the measurements of the response curves of a quartz crystal oscillator under a frequency sweep of the acoustic shear mode excitation applied across the crystal.^[28-31]

In this study, we dropped 100μl DNA solution (1 μM) on the gold coated quartz crystal surface and let it absorb overnight. Then the DNA structures were immobilized on the gold electrode by thiol group and the surface was rinsed with the PBS buffer for several times to remove the unabsorbed DNA molecular. Then the quartz crystal was installed in the flow cell, and the PBS buffer with pH 8.5 flowed into the liquid cell. Afterwards, after the frequency shift and dissipation factor reached a stable value, we left it for about 10 minutes, and then injected the PBS buffer of pH 4.5. The circle is repeated for several rounds.

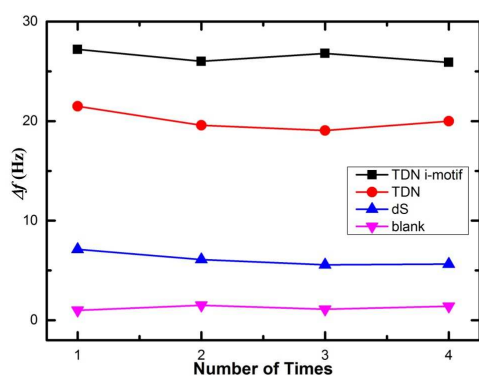


Fig.3./ Difference between the frequencies measured at pH4.5 and pH8.5 by the quartz crystal resonator. For DNA nanostructures of (■) TDN i-motif, (●)TDN, (▲)DNA double helix and (▼)blank.

The frequency of the quartz crystal was switched rapidly in a limited range after the buffer with different pH was injected into the flow cell (Fig.S1). Compared to the blank surface, all the three DNA films on the solid liquid interface are affected by the pH change (Fig.3.). The observed frequency shift is indeed introduced by the specific conformational changes of the DNA structures. Meanwhile, more complex the absorbed DNA structure is, larger the frequency change caused by the change of the buffer pH will be ($\Delta F_{\text{TDN i-motif}} > \Delta F_{\text{TDN}} > \Delta F_{\text{dS}} > \Delta F_{\text{Blank}}$). This implies the alteration of properties of the DNA film absorbed on the gold surface, and all these changes are reversible in four circles.

According to the phenomenon we observed above, we notice that not only the orientation (fold or stretch) of the TDN i-motif structure immobilized on the gold surface can be modulated electrically to produce an “open and close” signal, but also the TDN and even ordinary DNA double helix can play as a power switch based on pH. As shown in Fig.4, It is the schematic of the conformation changes of the three types of DNA nanostructures due to the pH change. Here, if the i-motif structure is a motor DNA induced by the change of the solution pH without the need of injecting external energy, we can say that any DNA structure even DNA double helix will gain the intrinsic conformation change, to stretch up and shrink down due to the change of pH solution, like the function of an i-motif motor DNA.

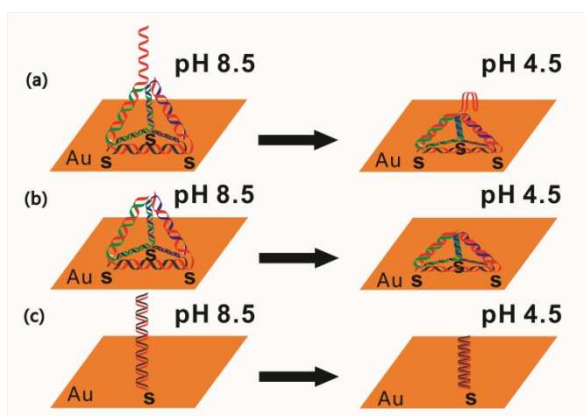


Fig.4./ Schematic drawing of DNA nanostructure modified gold-coated quartz crystal surface (a) TDN i-motif, (b) TDN, (c) DNA double helix.

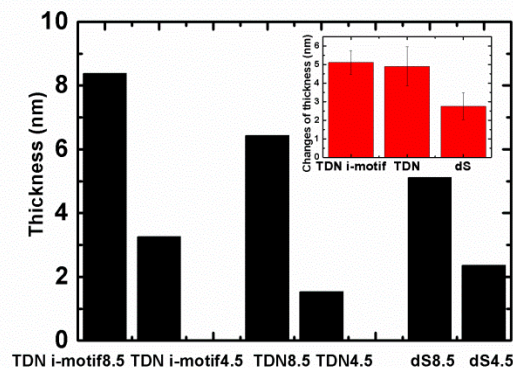


Fig.5./ Thickness of the DNA films on the gold-coated quartz crystal surface at pH4.5 and pH8.5. The inserted panel shows the change of thickness when pH goes from 4.5 to 8.5.

In order to further illustrate the structure changes of the DNA films on Au surface. We probed the thickness of these DNA films at pH4.5 and pH8.5. Four overtones (first, third, fifth and seventh) were used to model the thickness using the Voigt model implemented in the software (Q-Tools software 301version 2.1, Q-Sense, Sweden) (Eqs.S1, S2).^[32] By replacing the pH 4.5 with the pH 8.5 PBS, the thickness of the DNA film on the gold surface alters immediately. Fig.5 tells us the change of the thicknesses of the DNA films, the thickness of TDN i-motif film changes larger than that of the TDN film and the thickness of the double helix film changes the least. Replacing the pH 4.5 buffer with a pH 8.5 PBS increases the thickness of the TDN i-motif film from 3.3nm to 8.4nm and the thickness of the TDN film increases from 1.5nm to 6.4nm. Along with the growth of the complexity of the DNA structure modified on the surface, the change of the thickness becomes larger accordingly.

This agrees well with the experimental result, which says that the TDN i-motif structure reduces more in scale than the TDN. This is because the i-motif could fold itself from a stretch chain into a quadruplex structure which brings about 1.7nm variation in thickness. By contrast, the change of the thickness of the TDN structure is larger than that of the double helix. The DNA double helix prepared from pH 4.5 PBS was found to be 2.4nm thick, which is 0.9nm thicker than that of the TDN film. The thickness further increased to 5.1nm after the buffer pH turns to be 8.5, which suggests that the DNA double helix receives relatively less impact from pH than the 3D DNA structure. The results basically complement the theoretical height of these three structures, TDN (55bp) is about 5nm in height, and i-motif TDN is higher than 7nm because the i-motif film in PBS at pH 8.5 is lift up 2.6nm at least.^[27] The point lies on that the DNA double helix stretches unrestricted on the gold surface, and the TDN structure is more rigid than the double strand. As a result, the film of dsDNA may partially coil and is therefore thinner than the theoretical value.

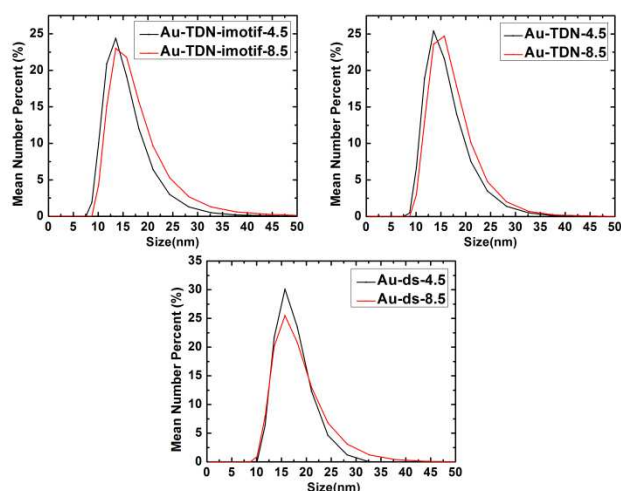


Fig.6/ Hydrodynamic size distribution profiles of the DNA nanostructures coated AuNP (a) TDN i-motif coated AuNP; (b) TDN coated AuNP; (c) DNA double helix coated AuNP

In addition, we connected these three DNA structures to gold nanoparticles to control the thickness of the DNA assembly layer on AuNP surface (Fig.S5). The surfaces of the gold nanoparticle are coated with the 3D DNA nanostructures via thiol group, and the coat acts as an absorbed film preventing the gold colloids from aggregation. As Fig.6 illustrates, a small increase of the hydration diameter observed when the identical structure was adsorbed on the surface of the gold nanoparticle. The thickness of the TDN film has about 1nm variation during the pH change, which is approximately 3nm for TDN i-motif at pH 8.5 and pH 4.5. It is smaller than that on the quartz crystal surface. That because the 3D DNA modified surface of a gold nanoparticle performed the random Brownian motion in the gold nano colloid. This motion may weaken the sensitivity of the 3D DNA structure and the influence of pH as well.

Considering the limitation of the dynamic light scattering method, such a small variation of the hydration diameter is in the range of the measurement error and does not change the main result. On the other hand, this method is undoubtedly promising because of its ability to control the thickness of the DNA assembly layer on AuNP surface, which can be employed in the field of the pH-controlled nanoswitch.

As a matter of fact, the 3D DNA structure undoubtedly varies as the function of pH. On the other hand, as a new generation of drug delivery vehicle, it has been found that the drug release was dramatically increased by lowering the pH from neutral to acid.^[33] Now, our study shows the pH-dependence of shrink and stretch of the 3D DNA structure on the solid liquid interface, it opens a new way to reveal the mechanisms of the drug release.

Conclusions

The structure changes of the DNA complexes near the solid-liquid interface is one of the most important and fundamental aspects of nanoscience, enabling a diverse range of applications in biological and physical disciplines. We monitored the stretch and the shrink of the TDN structure and the i-motif connected TDN at pH8.5 and pH4.5, and we found that not only i-motif could change its structure when pH changed, but also the TDN

even the DNA double helix changed their structures as well.

Notes and references

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[‡] Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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