Communication

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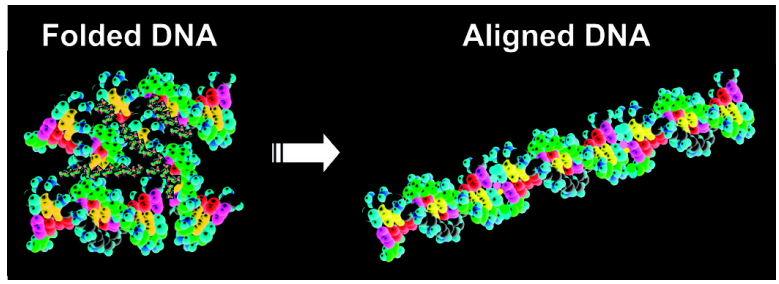

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High-density DNA Alignment on an Au(111) Surface Starting from Folded DNA

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A variety of DNA alignment processes on dielectric substrates, such as mica[1] and polymers,[2] has been developed for thorough studies of DNA properties.[3] Applications of an electric field,[4] surface tension,[5] and tiny tips[6] have contributed significantly to DNA alignment. Self-assembly of nanomaterials along this aligned DNA[7] is an established process for fabricating various metallic[8] and superconductive[9] DNA-templated nanowires.[10]

Recently, high-density uniform DNA alignment on metal substrates has been in high demand for creating sensitive DNA devices.[11] (See Supporting Information for a solution sensor using selectivity and sensitivity of DNA.) In particular, single DNA alignment (not bundled DNA alignment) directly on metallic substrates without using a self-assembled monolayer (SAM) or excess DNA buffer is required for sensitive sensors.

However, the interaction between DNA and metallic substrates, a complicated function of the buffer concentration and temperature, often causes DNA aggregation. In general, DNA can only be aligned three-dimensionally at a certain angle on a metal substrate in a solution with an appropriate solution pH.[3] This problem has historically prohibited the two-dimensional formation of high-density, uniform DNA alignment on metallic substrates.

This study develops a self-sensing DNA alignment process starting from folded DNA to achieve high-density, uniform two-dimensional DNA alignment directly on an Au(111) surface. The initiation of the alignment from folded DNA yields an unexpected benefit. The folded DNA (Figure 1a), which is generally considered unfavorable for the subsequent alignment process (Figure 1b,c), plays a critical role in avoiding DNA aggregation and aligning DNA directly on an Au surface at the greatest density and quality with excellent repeatability. This process will contribute significantly to creating selective and sensitive sensors with DNA on metallic substrates such as Au or Pr.[11]

The DNA sequence between the 5211th and 5510th base (~100 nm) in the λ-DNA was selected and thiolated using alkyl group (C_6H_{12}) at the 5′-end for this study. The alkyl group with hydrophobicity was used to avoid being included in the hydrophilic folded DNA and to keep the thiol group active. The outline of this DNA sequence is as follows (see Supporting Information for the detailed sequence and structure).

Sense sequence 5' – HS – AGGCTGCAGT ~ ATGGGGATCC – 3'
Antisense sequence 3' – TCCGACGTCA ~ TACCCCTAGG – 5'

This 300 bp of thiolated DNA dissolved in TE buffer was purified using the Boom method[12] and was stored in DNase-free water to fold the DNA (see Supporting Information for the distribution of DNA diameter in solutions). Two different concentrations of folded-DNA solution, 3.5 and 100 ng/μl, were prepared for this study. Forty microliters of this 3.5 or 100 ng/μl -DNA solution was sampled on a cleaned Au(111) surface in the trough, as illustrated in Figure 1d. The DNA solution was left for ten minutes so that thiol on the DNA could chemically interact with the Au surface. These preparations were conducted at 25 °C in 39% humidity.

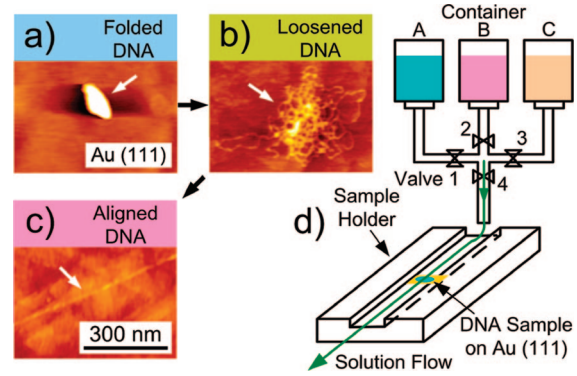

Figure 1. Apparatus and DNA alignment process starting from folded DNA on an Au(111) surface. White arrows indicate folded DNA (a), loosened DNA (b), and aligned DNA (c). DNA: λ-DNA.

The DNA solution on the Au surface was rinsed off using either DNase-free water or 45 mM of NaCl buffer flowing at 0.4m/sec for 35 s, as illustrated in Figure 1d (see Supporting Information for the detailed experiment setup). The 25/75 ethanol/DNase-free water flowed to the Au surface at a velocity of 0.4 m/sec for 35 s to rinse off excess NaCl buffer when it was used. These procedures were applied to both the 3.5 and 100 ng/μl concentrations of folded-DNA solution on the Au surface in the trough.

The Au samples were dried overnight in a desiccator at 1.6×10^4 Pa (0.158 atm) to permanently stabilize the aligned DNA. The stability of surface-bound DNA is important not only to observe it with AFM but also to apply further treatment with diverse solutions to activate and hybridize the DNA. The Au surfaces were observed with AFM (AFM: NanoScopeIIIa Tapping Mode, Digital Instruments/Veeco, Probe: Phosphorus-doped Si, ~50N/m spring constant, Model RTESP).

The results of the AFM observations are presented in Figure 2. Figure 2 panels a and b present images obtained after DNase-free water flowed onto the 3.5 ng/μl (Figure 2a) and 100 ng/μl (Figure 2b) of folded-DNA samples on the Au surface in the trough. Figure 2 panels c and d display images obtained after 45 mM NaCl buffer followed by 25/75 ethanol/ DNase-free water flowed onto the 3.5 ng/μl (Figure 2c) and 100 ng/μl (Figure 2d) of folded-DNA samples on the Au surface in the trough. Figure 2 panels a and b reveal that, on average, 7.9 and 237.2 folded DNA adhered to the 1 μm^2 Au surfaces. Figure 2 panels c and d illustrate 6.5 and 216.4 DNA (on average) were aligned on the 1 μm^2 Au surface along the direction of the flow (white arrows). The height of DNA in Figure

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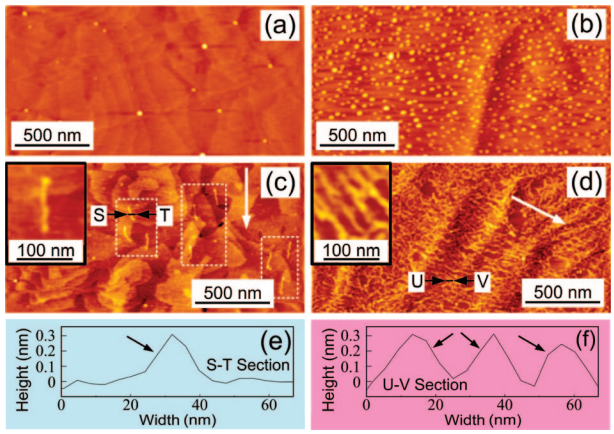

Figure 2. Self-sensing DNA alignment and AFM images of aligned DNA. The distributed, folded DNA was stimulated to align only when an appropriate solution flow was applied. Magnified images of aligned DNA are inserted at the left corners of panels c and d. DNA: thiolated 300 bp DNA.

2c was 0.32 nm on average and the root-mean-square (rms) of the DNA height in Figure 2d was between 0.3 and 0.4 nm, as indicated by their cross-sections in Figure 2 panels e and f (black arrows).

The AFM images in Figure 2 clearly show that the folded DNA on the Au(111) surface could be stimulated to align only when appropriate buffer flow was applied. The folded DNA dissolved in DNase-free water on the Au surface in Figure 2a could not be aligned by the application of 0.4 m/sec of DNase-free water. In contrast, Figure 2c clearly indicates that the folded DNA dissolved in DNase-free water on the Au surface could be aligned when 45 mM of NaCl buffer flowed at a velocity of 0.4 m/sec. The same results were obtained when using the different DNA concentration, as presented in Figure 2 panels b and d. The concentration of the DNA solution used in those figure panels was 28.5 times greater than that used in Figure 2 panels a and c.

Interestingly, the folded DNA in Figure 2b was distributed almost uniformly on the Au surface at a high density (237.2/μm^2 on average) without aggregation. This uniform high-density distribution of folded DNA apparently resulted in high-density DNA alignment (216.4/μm^2 on average) without tangling, as illustrated in Figure 2d. This high-density uniform DNA alignment on the Au surface had not occurred before we started sampling from folded DNA followed by the self-sensing alignment process using the appropriate buffer flow (see Supporting Information for aggregated DNA). This self-sensing DNA alignment starting from folded DNA was useful to avoid DNA aggregation particularly when the concentration of the DNA solution was increased, as indicated in Figure 2 panels b and d. However, it will be difficult to align longer DNA such as tens of micrometers without tangling with neighboring DNA if the DNA concentration is increased.

Detailed study of DNA conformation as a function of NaCl concentration revealed that the 300 bp of thiolated DNA was folded when the concentration was less than 3 to 5 mM and was aligned when it was more than 50 mM (see Supporting Information for the detailed results).

The negative O^- in phosphodiester plays a critical role in folding DNA at low NaCl concentrations less than 3 to 5 mM. In this solution, O^- in phosphodiester would share Na^+ ions by folding DNA to reduce electrostatic energy around O^- as depicted in Figure 2 panels a and b. This negative O^- in folded DNA also plays an important role in generating a repulsive interaction among folded DNA, enabling the folded DNA to distribute uniformly on an Au surface even at high density, as presented in Figure 2b. When the NaCl concentration is increased to 50 mM or more, O^- in folded DNA interacts with additional Na^+ ions by loosening DNA to reduce electrostatic energy around O^- more than by sharing Na^+ ions. The momentum of buffer flow combined with its surface tension[5,13] aligns this electrically loosened DNA along its streamline, as presented in Figure 2 panels c and d (white arrows).

The DNA conformation between 5 and 50 mM of NaCl solution was a mixture of folded, loosened, and aligned states, and the ratio of aligned DNA to folded increased with increasing NaCl concentration (see Supporting Information for the DNA conformation at this region). This is reasonable from the aligning mechanisms estimated in the previous paragraph. The application of a Fourier series to this region provided the following interesting interpretation of DNA conformation. Various DNA conformations are formed in a NaCl solution, which consists of NaCl solutions whose inverse concentrations and statistical weights are decided by the terms and coefficients of the series (see Supporting Information for a detailed Fourier analysis).

In summary, we developed a self-sensing DNA alignment process starting from folded DNA to achieve high-density, uniform, two-dimensional DNA alignment on an Au(111) surface. We demonstrated that folded DNA played a critical role in avoiding DNA aggregation and distributing the DNA uniformly on an Au(111) surface at the greatest density and quality ever attained. We also verified that folded DNA distributed on an Au surface can be stimulated to align only when appropriate buffer flow is applied. If DNA conformation is two-dimensionally reversible from folded to aligned as a function of buffer concentration on metal substrates, this will be a fundamental technology for creating dynamic self-sensing DNA sensors or switches.

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Supporting Information Available: Supporting Information is available free of charge via the Internet at http://pubs.acs.org.

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