学位論文の要約

題目　Design and Evaluation of DNA Nano-devices Using DNA Origami Method and Fluorescent Nucleobase Analogues
（DNA Origami 法および蛍光性核酸類縁体を用いた DNA ナノデバイスの設計と評価）

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General introdution

DNA is well known as the career of genetic information. Nucleic acid molecules can be utilized as excellent pieces for constructing nano-size structures and devices by using their unique structural motifs, physical robustness and self-assembling features. For constructing desired structure, DNA nano-technologies require elaborate designs and oligonucleotides. The establishment of DNA solid phase synthesis method enables us to readily prepare oligonucleotides which have desired sequences and dramatically advanced DNA-nanotechnologies. In this study, we have constructed a DNA origami tubular structure using a novel method, observed DNA-protein complex formation at single molecule-level using DNA origami template, synthesized and utilized a new emissive nucleoside analogue.

Chapter1

Helical DNA Origami Tubular Structures with Various Sizes and Arrangements

We developed a novel method to design various helical tubular structures using the DNA origami method. The size-controlled tubular structures which have 192, 256, and 320 base pairs for one turn of the tube were designed and prepared. We observed the formation of the expected short tubes and unexpected long ones. Detailed analyses of the surface patterns of the tubes showed that the short tubes had mainly a lefthanded helical structure. The long tubes mainly formed a righthanded helical structure and extended to the directions of the double helical axes as structural isomers of the short tubes. The folding pathways of the tubes were estimated by analyzing the proportions of short and long tubes obtained at different annealing conditions. Depending on the number of base pairs involved in one turn of the tube, the population of left-/righthanded and short/long tubes changed. The bending stress caused by the stiffness of the bundled double helices and the non-natural helical pitch determine the

structural variety of the tubes.

Chapter2

Single Molecule Visualization and Characterization of Sox2−Pax6 Complex Formation on a Regulatory DNA Element Using a DNA Origami Frame

We report the use of atomic force microscopy (AFM) to study Sox2−Pax6 complex formation on the regulatory DNA element at a single molecule level. Using an origami DNA scaffold containing two DNA strands with different levels of tensile force, we confirmed that DNA bending is necessary for Sox2 binding. We also demonstrated that two transcription factors bind cooperatively by observing the increased occupancy of Sox2−Pax6 on the DNA element compared to that of Sox2 alone.

Chapter3

Development of a Visible Nanothermometer with a Highly Emissive 2′-*O*-Methylated Guanosine Analogue

We have synthesized a fluorescent base analogue, 2-aminothieno[3,4-d]pyrimidine based G-mimic deoxyribonucleoside, 2'-OMe-thG, and investigated its photophysical properties and DNA incorporation. The 2' methoxy group of 2'-OMe-thG effectively induces the Z-form DNA. Finally we have achieved to construct nanothermometer based on the B-Z transition of DNA using 2'-OMe-thG.

Chapter4

Development of Distance and Orientation Controlled FRET System Using Emissive dG-dC Analogue Pair

We report the new type of Förster Resonance Energy Transfer (FRET)-pair using isomorphic nucleobase analogues. This FRET-pair consists of thdG[1-3] as an energy donor and the tC, [4] 1,3-diaza-2-oxophenothiazine, as an energy acceptor. The designed FRET-pair successfully shows distances of one turn of the DNA duplex as the difference in color. A combination of oligonucleotides containing the thG-tC FRET-pair at chosen positions enables to accurately distinguish the distance between donor and acceptor by using fluorescent spectra. In addition, this FRET-pair could form Watson-Crick base pair between thG and tC. This new FRET-pair based on emissive nucleobase analogues will have a great advantage in the study of DNA super organizations.