（ 続紙 1 ）

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論文題目	Artificially controllable nanodevices constructed by DNA origami technology: photofunctionalization and single molecule analysis （DNAオリガミ法を使った操作可能なナノディバイスの構築：その光機能化と一分子観察）		

（論文内容の要旨）

DNA molecules act as building blocks for assembling nanoscale biometerials by exploiting high specific recognition and excellent biocompatibility. DNA nanotechnology has already achieved great progress since the emergence of DNA origami technology. Based on this convenient method, various kinds of DNA nanodevices have been developed for multiple applications. In this thesis, it was mainly discussed about the photofunctionalization of DNA nanostructures in programmed patterns and direct observation single molecule behaviors in real time based on DNA nanoscaffolds system by high-speed AFM.

(1) Single-molecule visualization of switching behaviors in the DNA nanoframe system by using different kinds of molecular switches.

Here DNA based molecular switches: photoresponsive oligonucleotides and K^+-regulated G-telomeric repeats were introduced separately into frame-shaped DNA nanostructures. First a pair of azobenzene modified pseudocomplementary oligonucleotides (Azo-ODNs) were tethered into two parallel dsDNAs by disulfide coupling and then hybridized with a vacant DNA frame. As a result, the two parallel dsDNAs were associated at the central position in an X-shape because of the *trans*-form of azobenzene molecules. After the UV light irradiation, the two dsDNAs were dissociated back to the parallel-shape because of the *trans*- to *cis*-photoisomerization of azobenzene moieties. The hybridization and dissociation of Azo-ODNs was directly visualized using high-speed AFM by observing the movement and global change of two dsDNAs placed in the DNA frame. Using UV and visible light irradiation, the performance of hybridization/dissociation were reversibly confirmed both in solution and mica surface.

Furthermore, Azo-ODNs and two G-telomeric repeats were introduced together into another similar nanoframe system separately in which carrying three parallel dsDNAs. A nanoframe-shaped platform was constructed for visualization dual-switching behaviors corresponding to “State-transition” in real time by high-speed AFM. Photoirradiation and K^+ were used as input stimuli to switch the interaction among three dsDNAs in a logical manner. Three states were resulted and summarized: Association State-1 (Azo-ODNs associated), Relaxation State (both Azo-ODNs and G-telemeric moieties were free) and Association State-2 (G-quadruplex formed). As a result, a two-step cascading tranformation reaction was successfully performed from photoinduced dissociation and G-quadruplex formation in bulk solution and furthermore a series of dual-switching logical behaviors indicated by conformational change were

reversibly observed using high-speed AFM.

(2) Construction of photocontrollable DNA nanostructures in programmed patterns and direct visualization of dynamic assembling/disassembling process.

A hexagon-shaped DNA origami was designed and constructed as assembling unit. Azo-ODNs here were employed as photoresponsive linkers to control the assembly and disassembly of programmed hexagonal oligomers in various patterns. It was demonstrated that a distinctive photo-regulated self-assembly method for organizing defined regular or irregular DNA architectures composed of different numbers of hexagon units. Here the assembly and disassembly of the oligomers can be regulated reversibly under photoirradiation in a wavelength-dependent manner. Taking hexagonal dimer as model, the reversible assembling was confirmed by agarose gel electrophoresis. And also from fluorescence on/off results of dimers, the assembling/disassembling can be regulated reversibly over ten cycles by switching between UV and visible light. By using different patterns of assembling units, linear and curved hexagon-oligomers without facing control was obtained. Moreover, by adjusting the numbers and the positions of Azo-ODNs in the hexagonal units, the specific nanostructures such as curved and ring-shaped structures with face controlling were successfully constructed.

To realize the direct visualization of assembling/disassembling between DNA origami structures, a predesigned hexagonal dimer using same Azo-ODNs as linkers were investigated here. The observation relies on controlled interactions between the lipid bilayer on mica surface and cholesterol moieties introduced to the hexagonal unit. The hexagonal dimers absorbed on the bilayer surface were dissociated into two monomer units after introducing the UV irradiation. And then the dimer was formed again by switching to the visible light irradiation. These reversible dynamic processes were directly monitored using high-speed AFM.

In summary, combining precise manipulation with various kinds of functionalization of different molecules, DNA origami has gradually become a useful tool for the investigation of chemical/biochemical interactions in defined nanospace. Moreover, it is also shows the possibility of DNA nanostructures acting as nanodevices for the applications in various nanosystems.

（続紙 ２ ）

（論文審査の結果の要旨）

自然界に見られるような、自己集合を基にしたボトムアップ型のナノテクノロジーは現在、急速な勢いで進展している。また、外部刺激に応答して動作するナノスケールのデバイスは、新規材料や医療など様々な応用が期待できる。分子のプログラムに従った高度なナノスケールの構造体を設計・構築する上でDNAは非常に有用な素材である。DNAオリガミ法の確立によって、設計・構築した構造体に部品となる機能性分子を自由に配置することも可能となった。しかしながら、これまで外部からの制御によって1個の分子を正確に操作し、可視化する技術は確立していなかった。本論文において、申請者は、1個の分子を操作するために光反応を用い、DNA構造体を使ってその動きを高速原子間力顕微鏡（AFM）によって1分子可視化し、DNA構造体の集合や解離を人為的に操作することに成功した。

光異性化するアゾベンゼンを含むDNA鎖は紫外光(UV)及び可視光(Vis)を照射することで2本鎖の解離と形成を制御することが名古屋大学の浅沼らの研究で明らかとなっている。この光応答性DNAをDNAフレームと名付けたナノ空間を持つDNA構造体の内部に導入した。これらの光応答性2本鎖DNAはUV照射で解離し、Vis照射で再度2本鎖を形成することが確認でき、高速AFMで走査しながら光を照射することで2本鎖DNAの形成と解離が1分子のスイッチングの動作として可視化できた。また、2つの分子スイッチ、光応答性DNAとグアニン4重鎖を形成するDNA鎖を1つのDNAフレームに入れ、1分子操作を行った。グアニン4重鎖を形成する条件のカリウムイオン存在下で、UV照射を行うと、光応答性2本鎖からグアニン4重鎖に構造がスイッチングすることが実時間で可視化できた。一方で、DNAナノ構造体の集合と解離の操作方法も確立した。6角形のナノ構造体の外辺に光応答性DNAを導入し、2量体と単量体の集合と解離を高効率で実現した。この方法を用いると直線状や環状などの集合体を自由に構築でき、光操作によって集合体の解離や形成をプログラム的に制御することに世界に先駆けて成功した。

以上、本論文では、外部刺激応答性分子をナノ構造体に導入したスイッチングデバイスを開発した。そして、そのメカニカルな分子の動きを高速AFMによって1分子可視化する方法を確立した。これらの申請者の成果は、DNAナノ構造体、光操作、高速AFMといった独創的な方法を用いることで成し遂げられたものであり、従来の方法では技術的になしえなかったものである。これらの成果はナノサイエンスの世界で画期的であり、大きなインパクトを持つ。これらのスイッチングデバイス構造体はドラックデリバリーのキャリアーや細胞表面でのレセプターなど多方面に使用可能であり、将来の応用性も高い研究である。よって、本論文は博士（理学）の学位論文として価値あるものと認める。また、平成２６年１月１４日論文内容とそれに関連した口頭試問を行った。その結果合格と認めた。

要旨公表可能日：　　年　　月　　日以降