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Light Sensitization of DNA Nanostructures via Incorporation of Photo-Cleavable Spacers†

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Using light irradiation as a trigger, large-scale structural reconfiguration of DNA nanostructures is demonstrated. We incorporated photo-cleavable spacers at strategic locations within the short oligonucleotide strands connecting adjacent helices within a DNA origami sphere, and then used light to transform the sphere into two tethered hemispheres.

Self-assembly of DNA represents a powerful method of creating intricate two and three dimensional nanostructures.1,2 Recent advances in DNA nanotechnology have enabled the efficient fabrication of a large diversity of nanoscale objects in only one step.3-16 Utilization of these nanostructures for biomedical applications would be greatly advanced if they could undergo dynamic reconfigurations in response to specific stimuli.17-19 Several studies have demonstrated that chemicals such as oligonucleotides,7,20-23 bioactive small molecules,24,25 or acid26 can be used effectively to trigger such transformations. For example, chemicals have been used to induce DNA devices to travel on patterned surfaces,27,28 undergo topological reconfiguration,21 or expose encapsulated cargo.22,24,25 Light irradiation represents another attractive stimulus because it can be controlled with high spatial and temporal resolution without the limitations associated with chemical diffusion. Recent studies have demonstrated the feasibility of attaching light sensitive oligonucleotides to the surfaces of DNA origami allowing the assembly and disassembly between multiple 2-D DNA origami particles29 or the release of protein cargo tethered outside of a 3-D origami.30 The applications for dynamic DNA nanotechnology would be further expanded if light could be used to reconfigure the structures of DNA nanoparticles themselves, in addition to altering their connectivity to external factors.

Here we present a novel strategy to trigger large-scale structural reconfigurations within a DNA nanostructure via the strategic incorporation of photo-cleavable spacers as part of the structural component of a DNA origami nanoparticle. We designed an origami sphere containing photo-labile spacers within the crossovers (photo-crossovers) at desired locations without interfering with the assembly of the nanostructure. Upon light irradiation, these origami spheres were transformed to two tethered hemispheres. Because crossovers are commonly used as a structural component for connecting adjacent helices in DNA origami designs, the strategy presented here can be broadly applied to a variety of DNA nanostructures. In addition to the superb spatiotemporal precision of light irradiation, light is also capable of penetrating into environments inaccessible to chemical stimuli such as the interiors of nanoparticles. The ability to trigger large-scale structural transformation using light, in addition to chemical controls, will greatly facilitate the applicability of dynamic DNA nanotechnology in various biomedical applications.

Fig 1. (a) Schematic diagram of the light reconfigurable DNA sphere. The cylinder represents the scaffold DNA strand, and the top and bottom hemispheres are shown as white and blue respectively. The excess scaffold is omitted. Insert: The red helices represent the crossover strands that connect the top (white) and bottom (blue) scaffold helices. (b) Depiction of the separation of adjacent helices that would result when photo-crossovers were cleaved with light.
Figure 3. Light-triggered transformation of the DNA sphere into two hemispheres. (a) Schematic depiction of the structural reconfiguration of the sphere upon exposure to light. (b) Fluorescent image of SYBR Safe stained 1.8% agarose gel showing successful photo-reconfiguration of the DNA sphere. L = 1kb ladder, m13 = m13 DNA scaffold, 1 = Closed sphere containing o-nb photo-crossovers, 2 = Sphere containing o-nb photo-crossovers after 10 min light irradiation. TEM images of the nanostructures before (c) and after light irradiation (d). Scale bars are 100 nm (zoom out) and 50 nm (zoom in).

We first investigated the structural transition occurring in the absence of any crossover that holds the two rings together at the sphere’s equator. Because our structure does not utilize the entirety of the m13 sequence, we utilized the unfolded excess scaffold DNA as a design criterion to maximize the structural reconfiguration and positioned two equal length portions of unfolded DNA between the two largest rings at the equator of the sphere (Fig. 2a-c). We created spheres with and without all equator crossovers, as illustrated in Fig. 2a. When all equator crossovers were included in the reaction mixture, uniform spheres were observed in transmission electron microscopy (TEM) images (Fig. 2d). The particle diameters and DNA density were in good agreement with the design. Next, we created structures in the absence of any equator crossovers to mimic the complete cleavage of all equator crossovers, and observed well separated hemispheres rather than closed spheres. The separated hemispheres were up to ~100 nm apart (Fig. 2e), a length consistent with the predicted 106 nm length of the 311 base pairs of DNA located between the two hemispheres. Interestingly, the DNA strands connecting the two hemispheres were clearly visible in the TEM images. Although these strands represents only a small percentage of the overall m13 DNA length (8.6%), this linearized portion can create a separation distance larger than the diameter of crossovers (Fig. 1a insert, red strands). We hypothesized that if photo-crossovers were positioned throughout the structure’s equator, light irradiation would sever the covalent connections between the two hemispheres leading to a large structural reconfiguration. For the photo-cleavable linkage, we used ortho-nitrobenzyl (o-NB) groups which are ideal for DNA nanotechnology because they are commercially available and can be readily incorporated at desired positions within a crossover (Fig. 1b). Although the size of o-NB groups in our photo-crossovers is bigger than the phosphate bonds in conventional crossovers, structural interference is expected to be minimal when they are introduced into all crossovers within one plane of the sphere.
the hemispheres/spheres. While the unfolded excess scaffold DNA has not been broadly used as a design consideration in DNA origami (for an exception, see reference 34), our results highlight that the excess DNA can have important consequences in dynamic DNA nanotechnology.

We next minimized the number of equator crossovers required to produce fully closed spheres so that fewer o-NB groups would need to be photolyzed to initiate a structural reconfiguration upon light irradiation. We discovered that when only 3 of the 9 crossovers were used (2, 5, and 8 in Fig. 2a), closed spheres formed predominantly (Fig. S4). We then assembled light sensitized spheres containing photo-crossovers in the 3 identified locations, and found that closed spheres formed equally well with photo-crossovers compared to those formed with unmodified crossovers, suggesting that o-NB photo-spacer incorporation does not interfere with self-assembly despite the increase in distance they introduced between connected helices (Fig. S1, S5).

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33 To achieve maximal separation between the hemispheres using the Tethered Design, staple sequences complementary to the excess scaffold must be included.
35 www.idtdna.com/Site/Catalog/Modifications/Product/1707