Click and photo-release dual-functional nucleic acid nanostructures†

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We functionalize nucleic acid nanostructures with click chemistry (for attachment of cargos) and a photocleavable linker (for release). We demonstrate cargo attachment using a fluorescein dye and release using UV trigger from an RNA three-way junction, a DNA star motif and a DNA tetrahedron. Such multifunctional nucleic acid nanostructures have potential in targeted drug delivery.

Nanoscale structures built from DNA have potential applications as nanocarriers for targeted drug delivery.1 Site-specific attachment of guest molecules and controllable release are two parameters required for such drug delivery carriers. For attachment, scientists have used intercalation,2 crosslinking,3 ligand-receptor interactions4 and hybridization.5 For release, DNA nanostructures can be stimulated to undergo a conformational change by molecular triggers such as other nucleic acids,6 small molecules,7 enzymes,8 or environmental factors like pH.9 Adding to this list, a variety of chemical groups can be incorporated into oligonucleotides to enable control by light. Previously, researchers have used light to control DNA branch migration reactions,10 operate a DNA nanomachine,11 perform site-specific cleavage on DNA origami,12,13 reconfiguration of nanostructures,14–16 and to release molecules from DNA nanostructures.17,18 In this study, we show proof-of-concept dual functionality in nucleic acid nanostructures where we incorporate both attachment and release chemistries within a single nanostructure. As a model system, we attached a guest molecule (6-fluorescein azide, FAM) via click chemistry, and show release of the attached moiety using a photocleavable linker that is cleaved by UV light (Fig. 1). We demonstrate the versatility of our approach using three nucleic acid nanostructures that vary in size, dimensionality, and composition: an RNA three-way junction, a DNA motif (3-point-star), and a 3D DNA object (tetrahedron).

To create our designed nanostructures, we synthesized DNA and RNA strands containing both a 2′-O-propargyl (2′-OP) modified nucleotide19 and a photocleavable linker (PCL).20 The 2′-OP modified strand allows the attachment of an azido-modified pay load via click chemistry (Fig. 1a) while the PCL group allows light-triggered release of the cargo (Fig. 1b). We have earlier designed oligonucleotides with 2′-OP as functional units to attach moieties to DNA nanostructures.21 The PCL used in this study is commercially available and can be site-specifically incorporated into any DNA or RNA sequence. Further, the PCL is rapidly cleaved using near-UV while also leaving a 5′-phosphate that allows downstream processing of oligonucleotides and assembled structures. We validated clicking of the cargo (FAM) and UV-triggered cleavage of the strand (λ = 254 nm) using denaturing polyacrylamide gel electrophoresis (PAGE) (Fig. 1c and Fig. S1, S2, ESI†).

Using this strategy, we tested functionalization of three different nucleic acid nanostructures: an RNA three-way junction (3WJ), a DNA 3-point-star tile, and a DNA tetrahedron. The RNA 3WJ is part of the packaging RNA (pRNA) of the bacteriophage phi29 dsDNA packaging motor,22 previously shown to be very stable and a viable drug delivery vehicle.23 The 3WJ has three component RNA strands of similar lengths (Fig. 2a and Fig. S3, ESI†), one of which we modified near the 5′ terminal with both 2′-OP and PCL. We assembled the 3WJ using equimolar concentrations of each strand and confirmed its proper formation with and without the modification using non-denaturing PAGE (Fig. 2a and Fig. S3, ESI†). We found that the incorporation of the modified oligo in the 3WJ was lower than other structures, possibly due to its effect on junction formation (Fig. S3, ESI†). Stability of nucleic acid junctions increases with the length of the arms, and increasing the base pairing on each of the arms could lead to better assembly yields and minimize the effect of the modifications.

The second model system is a DNA 3-point-star tile (DNA tile), a three-fold symmetric DNA motif where each arm is a four-arm...
Having successfully assembled the structures with the modified oligonucleotides, we next tested light-triggered release of the fluorophore cargo with UV exposure. We exposed each of the three structures to UV (254 nm) for various times up to 16 min to achieve photocleavage of the PCL and measured the results using non-denaturing PAGE. Gel profiles showed decreasing FAM intensity of the nanostructure band with increasing UV exposure time and appearance of an additional band near the bottom of the gel corresponding to the released FAM (Fig. 2d–f and Fig. S6, ESI†). Native nanostructures without the modifications were not affected by UV exposure at 16 min (Fig. S7, ESI†). To estimate the rate of release, we quantified the intensity of the bands corresponding to FAM. For the DNA tile and tetrahedron, we observed more than 75% release taking place by 4 minutes of UV exposure, which is consistent with the efficiency we observed in single strand cleavage (Fig. 1c). Complete release of the FAM was achieved in ~16 min for the DNA tile and tetrahedron while 80% release was observed for the RNA 3WJ at the same time point.

As a step toward utility in drug delivery, we tested the stability of these nanostructures and the release mechanism in complex biological fluids to see if physiological conditions affect the nanostructures or its stimuli-responsive release. We incubated the DNA tile in fetal bovine serum (10% FBS) and synthetic urine and shined UV on the samples. Results showed that the nanostructure is stable in these biological fluids and UV-triggered release is also functional as compared to release in buffer (Fig. S8, ESI†). In FBS, full release was not achieved in 16 min, possibly due to the presence of other biological molecules in the serum. Further studies will establish the potential use of DNA-based structures in drug delivery, and their robustness to sustain in biological conditions.

Assembly of such DNA nanostructures is very sensitive to the size and position of modification. In the examples above, we inserted modifications toward the outside of the structures so that they do not interfere with assembly. To test the formation of structures with internal modifications, we synthesized component strands for the DNA tile and tetrahedron that position the 2′-OP and PCL modifications within the nanostructures on assembly. In the case of DNA tile, incorporation of modifications within DNA nanostructure did not significantly affect formation of the structure (Fig. S9, ESI†). However, when we used these tiles to assemble the DNA tetrahedron, we found that the assembly yields were lesser for the structure with internal modifications. The PCL is similar to an additional nucleotide in the structure and could thus affect the twist of the tile arms, resulting in destabilization of the assembly.

Our study compares different nanostructures based on size, dimensions (2D and 3D) as well as starting materials (DNA and RNA) being used as stimuli responsive nanostructures. The proof-of-concept for dual functionality was established on these nanostructures by using click chemistry as means of functionalization and PCL as the means of controlled release. The click strategy provides quantitative, robust, and at the same time facile ways to attach a payload onto nanostructures. The PCL provides non-contact control over payload release for a variety of nanostructures. Our strategy can be expanded to specific control over different parts of a nanostructure by using different
Use of UV as a trigger does pose some challenges and limitations including absorbance and potential damage of biological materials. The nitrobenzyl protective groups that remain after UV cleavage could also have a toxic effect, but this type of caging system has been widely applied in many biological systems. It is likely that the UV-released byproduct (nitrophenyl short oligos) might cause immune response; this aspect needs more systematic cellular studies measuring the overall efficiency and toxicity, which are currently undergoing in our lab. Future work may also focus on other wavelengths, especially those in the near-infrared (NIR) that have minimal absorbance by skin and tissue. With a number of wavelength-specific photoactive chemistries available, it could be possible to have DNA nanostructures that respond to multiple wavelengths. The programmability of such approaches coupled with the suite of chemical tools may one day enable complex functions such as selective release of drugs from multi-drug cocktails.

Research reported in this publication was supported by the NIH through NIGMS under award R35GM124720 to K. H. and R15GM124627 to J. S.

Conflicts of interest
The authors have no conflicts to declare.

References