JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
pubs.acs.org/JACS
Communication

Programming Dynamic Assembly of Viral Proteins with DNA Origami

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Cite This: https://dx.doi.org/10.1021/jacs.9b13773

ABSTRACT: Biomolecular assembly in biological systems is typically a complex dynamic process regulated by the exchange of molecular information between biomolecules such as proteins and nucleic acids. Here, we demonstrate a nucleic-acid-based system that can program the dynamic assembly process of viral proteins. Tobacco mosaic virus (TMV) genome-mimicking RNA is anchored on DNA origami nanostructures via hybridization with a series of DNA strands which also function as locks that prevent the packaging of RNA by the TMV proteins. The selective, sequential releasing of the RNA via toehold-mediated strand displacement allows us to program the availability of RNA and subsequently the TMV growth in situ. Furthermore, the programmable dynamic assembly of TMV on DNA templates also enables the production of new DNA−protein hybrid nanostructures, which are not attainable by using previous assembly methods.

Dynamic self-assembly is crucial in controlling the formation of naturally occurring complex biomolecular structures, such as cytoskeletal motors, transcription factors, and ribosomes. In biological systems, the dynamic self-assembly processes are regulated by the information flow between biomolecules (e.g., proteins and nucleic acids) at different stages.[1,2] De novo design of artificial nanostructures via biomolecular assembly has seen impressive growth in the last several decades.[3−7] Among various design paradigms, DNA nanotechnology has produced some of the most massive and complex nanostructures, largely due to the excellent programmability and predictability of the interactions between DNA strands.[8−12] Nevertheless, to explore the full spectrum of biomolecular assembly to improve the sophistication and functionality of artificial nanostructures, novel approaches for constructing nucleic-acid−protein hybrid assemblies and particularly dynamic assemblies are needed.

Recently, we developed a new approach for constructing nucleic-acid−protein chimeric nanostructures by programming the in situ tobacco mosaic virus (TMV) protein assembly on DNA origami templates.[13] The approach is based on the premise that the DNA template provides an addressable platform on which to anchor RNA strands consisting of the origin of assembly sequence (OAS),[14] which triggers the assembly of TMV proteins in situ. Here, we show DNA origami templates can be designed to dynamically control the assembly of TMV proteins, and new nucleic-acid−protein chimeric nanostructures can be constructed by programming the routes of the dynamic TMV assembly on DNA origami. Our new strategy uses a DNA origami template to arrange an RNA with a series of path points−single-stranded DNA strands protruding from the origami. These DNA strands are designed to bind to the specific segments of the RNA strand, which is captured on the DNA origami surface along the programmed route (Figure 1a). The OAS of the RNA is left free to initiate the TMV protein assembly. Our study revealed that the anchor

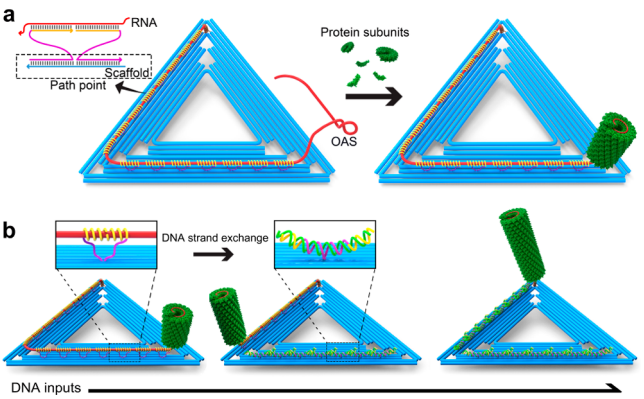

Figure 1. Schematic illustration of a programming RNA strand on a DNA platform for controlling protein dynamic assembly. (a) Arranging an RNA strand (red) on a DNA scaffold (blue) using extending strands on path points, each containing a binding region complementary to RNA and release-toehold region for controlled release of RNA via toehold-mediated strand exchange. The accessible overhang of the RNA region contains the OAS, which can trigger the formation of a short TMV-like rod. (b) Stepwise release of anchor points on demand by toehold-mediated strand exchange in the presence of a specific full-length complementary strand for guiding sequence-dependent dynamic assembly of the TMV protein complex.

DNA strands on DNA origami can also act as "locks" that stop the protein assembly when the TMV growth reaches the anchor points. This allows us to use toehold-mediated DNA

Received: December 21, 2019
Published: March 19, 2020

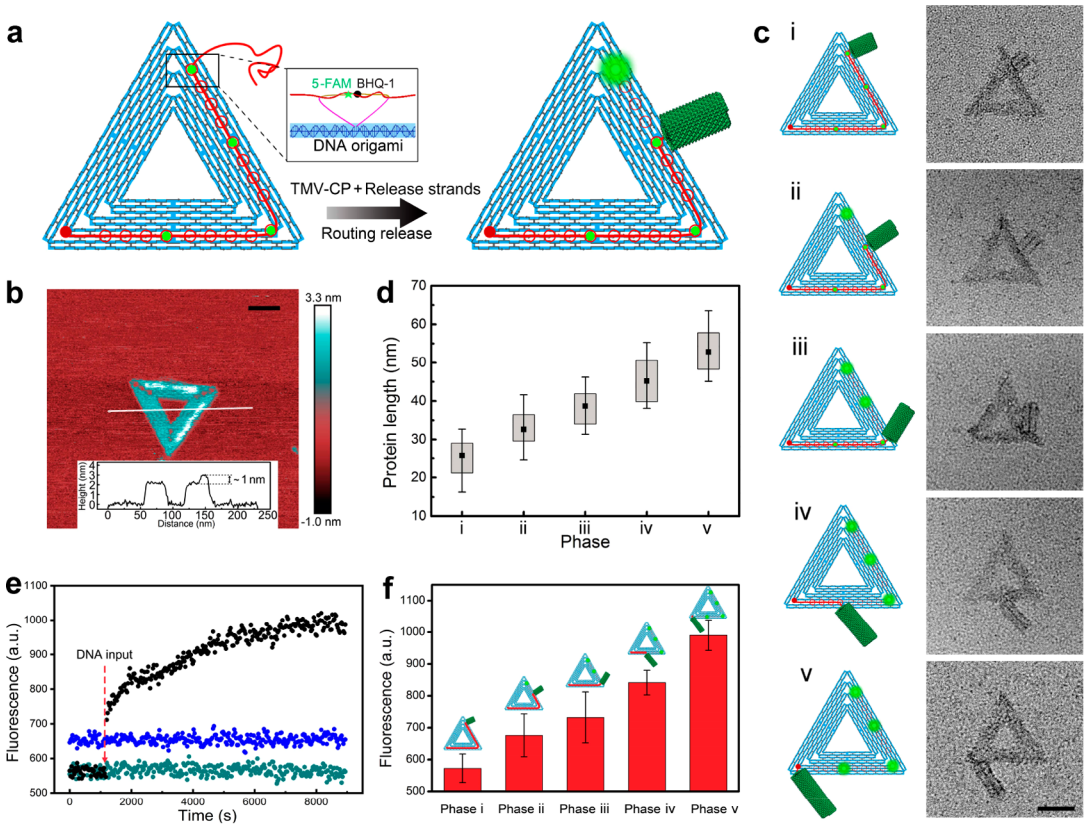

Figure 2. Routing the RNA sequence for the dynamic control of protein assembly. (a) Design of patterning an RNA strand (red) on triangular DNA origami (blue). Four fluorophore−quencher pairs (green dots) were set as checkpoints, in which 5-FAM and BHQ-1 were labeled on the DNA extremities (inset frame). (b) AFM of arranging an RNA strand on a triangular origami. Inset: Height profile of the white line. (c) Models and representative TEM images of phases from i to v corresponding to the design of fluorescence checkpoints in (a). (d) Boxplots for protein rod length analysis, with median values (black dots), 15/85% percentiles (gray boxes), and maximum/minimum values (whiskers) indicated. (e) RNA release kinetics monitored by fluorescence over time after the addition of 17 release strands (black). Samples before (blue) and after (dark cyan) binding of RNA were set as references. (f) Fluorescence values of the corresponding phases. Error bars represent the standard deviation. Scale bars, 50 nm.

strand displacement[15] to dynamically control the assembly of TMV on DNA origami: A toehold region is included in each anchor DNA strand, and the addition of a complementary DNA strand will release the RNA strand from the anchor DNA strand to enable assembly of TMV protein along the previously inaccessible RNA in a programmable manner (Figure 1b).

First, we used a triangular DNA origami as the platform to control the arrangement of a 1234-nt RNA strand on its surface (Figures S1 and S2). A pair of neighboring 40-nt capturer strands fully complementary to the 3′-terminus of the RNA were used for efficiently anchoring the 3′-end of the RNA. Additionally, 17 pairs of DNA strands were programmed on the origami surface to serve as removable anchor points for routing of the RNA (Figure 2a and Figure S3). Each path point consisted of two extended 29-nt sequences that were each complementary to a successive 13-nt domain of the RNA strand. The remaining 16 nt serve as the release-toehold region for the sequence-specific strand displacement for controlled RNA release. In order to monitor the capture and release of the RNA, we employed fluorophore−quencher pairs at the designated path points (Figure 2a and Figure S4). Specifically, one of the two 29-nt extensions was labeled with a fluorescent reporter dye (5-FAM) and the other with a quencher (BHQ-1).

Atomic force microscopy (AFM) was used to characterize the path-dependent binding of the RNA on the triangular DNA origami. We deleted the 16-nt flexible release-toehold region to increase the stiffness for better signal sensitivity for AFM detection.[16] The patterned RNA was visible as a ∼1 nm increase in height on the surface of triangular origami, revealing the successful routing of RNA along our designed pattern by binding to the path points (Figure 2b and Figure S5). Native agarose gel electrophoresis (Figure S6) and AFM imaging confirmed the high RNA binding efficiency onto the designed paths (Figures S7−S10). Furthermore, the RNA binding efficiency to the anchor points was also verified by using dye−quencher DNA strands (Figures S11 and S12).

Given sufficient TMV capsid proteins, the OAS on the 5′ overhang of the RNA strand triggered an autonomous assembly process, in which one subunit combined with three RNA nucleotides, resulting in the growth of a TMV-like protein rod.[17,18] The assembly reaction proceeded until the free RNA fragment was depleted;[19,20] thus it was suspended by locking onto the nearest path point. We controlled the stepwise addition of corresponding releasing DNA strands to precisely release the RNA sequences for subsequent assembly on demand. Therefore, the dynamic assembly of a protein complex could be exquisitely programmed on the designed route with high precision upon the input DNA information. We chose five typical moments, termed phases i to v, to verify the route-directed assembly process. The transmission electron microscopy (TEM) data obtained from the resulting sample

https://dx.doi.org/10.1021/jacs.9b13773
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revealed the dynamic control of protein assembly in the expected patterns (Figure 2c). As designed, the RNA packaging event occurred along the prescribed route on the DNA origami, thereby providing the high fidelity of information flow-directed dynamic assembly of the protein rod. More TEM images and yield analysis is included in Figures S13−S17. The increased length over the phases (i to v) agrees with the theoretically predictable growth of length from ∼27 nm to ∼54 nm, corresponding to the released RNA domains (Figure 2d and Figure S18). Using the fluorophore−quencher pairs positioned at four path points specific to the pauses at phases i, ii, iii, and iv (green dots in Figure 2a), we detected a reduction in fluorescence intensity owing to the binding of the RNA strand, which transformed fluorophore−quencher pairs from a flexible open configuration to a closed state for fluorescence quenching (Figure 2e, blue and dark cyan dots). According to the transition kinetics we observed, the addition of release strands transformed the closed state into an open state and freed the RNA via toehold-mediated displacement within 2 h (Figure 2e, black dots, and Figure S19). The fluorescence intensities gradually increased from phase i to v, further reflecting the controlled release of RNA for TMV protein assembly along our designed route (Figure 2f), in concordance with the TEM analysis.

In biology, dynamic biomolecular assembly usually produces delicate, nonequilibrium structures that are different from the thermodynamically favored products.[21] Similarly, the programmable dynamic assembly of proteins on DNA origami templates can be used to construct new nucleic-acid−protein chimeric nanostructures. In this work, we decided to assemble TMV inside of a DNA origami barrel. Such a chimeric structure would be extraordinarily difficult to construct using previous in situ TMV assembly along an RNA strand with only its end point anchored on the DNA origami barrel, because the RNA strand is likely to stay outside of the barrel during the TMV growth. However, by precisely arranging the path of the RNA onto the inner surface of the DNA barrel, we are able to guild the in situ dynamic assembly of TMV protein subunits into the confined space of a DNA origami barrel.

We engineered a 23-helix bundle (HB) DNA barrel origami with ∼34 nm inner diameter and ∼30 nm height (Figure 3a and Figure S2). The diameter of the TMV rod is 18 nm; thus this DNA design allows for easy diffusion of protein subunits into the barrel, as well as the final accommodation of one TMV rod. Figure 3a shows the purified DNA barrels by TEM imaging, some of which were laterally flattened on the TEM grid. As usual, we set a pair of 40-nt dangling strands that protrude out of the same position at one edge of DNA barrel to capture the last 80 nt of the 3′-terminus of the RNA strand. Next, path points were positioned on both the inner surface and the outer surface close to the edge on the other side, to guide the flexible RNA through the tunnel (Figure 3b and Figure S20). After the addition of TMV capsid subunits, we found a short TMV capsid rod formed at the location very close to the edge of the DNA barrel (Figure 3c), consistent with the position of the last path point we designed for anchoring. The resulting ∼26 nm long protein rod also agrees with the theoretical protein length by completely packaging the RNA overhang containing the OAS. We further opened the path points to release RNA and induce the growth of protein into the DNA barrel. We observed the formation of a protein rod in the pore of the DNA barrel (Figure 3d). The entrapped protein length is about 54 nm, indicating the successful

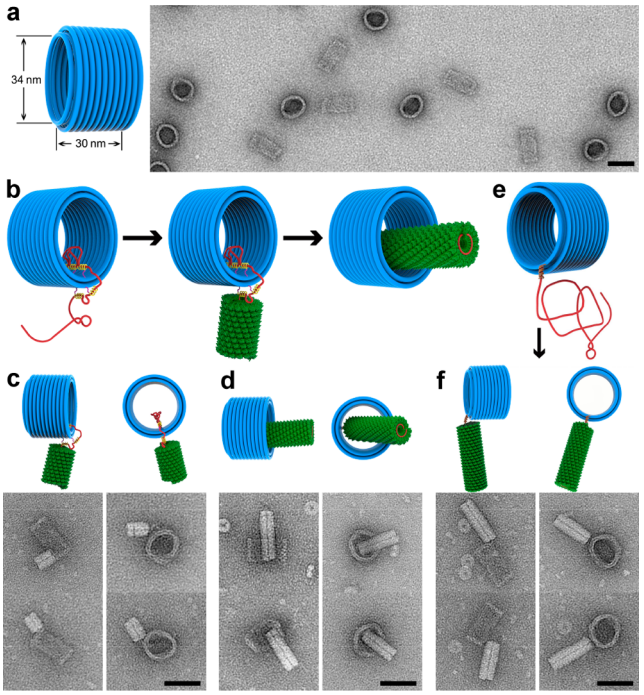

Figure 3. Programming the routing for inducing the assembly of a TMV-like rod into the 23-HB DNA barrel origami. (a) Model and TEM image of the DNA barrel origami. (b) Schematic drawing of routing of an RNA strand into the barrel-shaped DNA origami where release of the RNA strand regulates the assembly of the TMV rod through the DNA barrel. (c) Models and typical TEM images showing a short protein rod binding to the DNA barrel after the routing process of RNA. (d) Models and typical TEM images showing a protein rod threading the DNA barrel. (e) Model of an RNA strand binding to the edge of a DNA barrel. (f) Models and typical TEM images showing the growth of a protein rod on the outside of a DNA barrel without the information routing of RNA inside the DNA barrel. Scale bars: 50 nm. See Figures S21−S23 for additional TEM images.

packaging of the entire RNA strand (expect for the last 80 nt as the hybridization end). In addition, we also found shorter protein rods stuck in the DNA barrels, of which two ends tightly touched the barrel wall, thus preventing the following assembly (Figure S22). However, as expected, without the path points for the information routing process, the RNA tethered on the edge of the DNA barrel failed to grow into the DNA barrel channel by itself (Figure 3e,f). This is likely due to electrostatic repulsion and steric hindrance, resulting in the growth of a rod formation around 54 nm only outside of the DNA barrel structure.

In summary, our work demonstrates the regulation of information flow for dynamic viral protein assembly using DNA nanotechnology to program the informative genome-mimicking RNA sequence. This strategy enables precise control over protein assembly within the dynamic system including travel paths, positions, and assembly stoichiometries, which is of great importance for precisely constructing hybrid nanostructures with increased complexity and functionality. Furthermore, it provides an elegant approach to induce the assembly reaction in a confined space including interior cavity and tunnel of 3D DNA structures, by rationally dealing with the surface patterning of flexible RNA. Based on intricate DNA structures and multiple virus packaging mechanisms,[22−24] we expect to develop smart machines with various crosstalk

https://dx.doi.org/10.1021/jacs.9b13773
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

between DNA inputs and protein dynamic behaviors. We also envision our paradigm can encourage the use of programable DNA templates to control the accessible states of viral genomes for investigating the assembly mechanisms of other viruses.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.9b13773.

Experimental details, TEM and AFM images, and DNA design (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Qian Wang for assistance with the TMV virion. This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 21673280, 21425103, 21977112, 21703282, 21934007) and Chinese Academy of Sciences (Grant Nos. XDB32030200, 121E32KYSB20180021). Y.K. acknowledges the support of NSF Grants DMR-1654485 and ECCS-1807568 and Semiconductor Research Corporation (SRC) Grant 2018-SB-2836-B. The AFM images were collected on an AFM supported by National Institute of Health (NIH) grants R01GM084070 and 3R01GM084070-07S1 to Laura Finzi.

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https://dx.doi.org/10.1021/jacs.9b13773
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX