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Selective In Situ Assembly of Viral Protein onto DNA Origami

Kun Zhou,‡ Yonggang Ke,‡ and Qiangbin Wang†‡§*

‡CAS Key Laboratory of Nano-Bio Interface, Division of Nanobiomedicine and i-Lab, CAS Center for Excellence in Brain Science, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou 215123, China

§Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia 30322, United States

†School of Nano-Tech and Nano-Bionics, University of Science and Technology of China, Hefei 230026, China

Supporting Information Placeholder

ABSTRACT: Engineering hybrid protein-DNA assemblies in a controlled manner has attracted particular attention, for their potential applications in biomedicine and nanotechnology due to their intricate folding properties and important physiological roles. Although DNA origami has served as a powerful platform for spatially arranging functional molecules, in situ assembly of proteins onto DNA origami is still challenging, especially in a precisely controlled and facile manner. Here, we demonstrate in situ assembly of tobacco mosaic virus (TMV) coat proteins onto DNA origami to generate programmable assembly of hybrid DNA origami-protein nanoarchitectures. The protein nanotubes of controlled length are precisely anchored on the DNA origami at selected locations using TMV genome-mimicking RNA strands. This study opens a new route to the organization of protein and DNA into sophisticated protein-DNA nanoarchitectures by harnessing the viral encapsidation mechanism and the programmability of DNA origami.

Hierarchical assembly of biomolecules have been used to construct sophisticated nanoscale architectures. Particularly, hybrid assemblies integrating nucleic acids and proteins have attracted intense interest because the combination of the two essential biomolecules can lead to greater structural complexity and functionality.1 In recent years, DNA nanotechnology has achieved impressive developments, producing a variety of intricate structures via a range of rational design strategies based on the principle of complementary base pairing.2 Taking advantage of the sequence specificity, DNA origami nanoarchitectures provide spatial addressability for the precise positioning of guest proteins, and thus have enabled several applications including multi-enzyme cascades,3 nanoactuators,4 and as platforms for functional protein arrays.5 However, these methods for protein organization typically require chemical modification of proteins,6 which is likely to negatively impact the protein activities and render them inapplicable to in vivo systems. A few examples of non-covalent conjugation of proteins on DNA scaffolds have also been demonstrated by using biotin-streptavidin interaction,7 aptamers,8 and DNA binding proteins.9,10 Nonetheless, development of new strategies for in situ assembly of protein components is crucial for building DNA origami–protein chimeric complexes with precise control over the shapes and protein-DNA stoichiometry.

Here, we demonstrate a facile strategy to construct controllable DNA origami–protein chimeric complexes. Our method employs locally driven in situ assembly of virus coat proteins onto DNA origami in a highly organized fashion. Viruses composed of capsid and genome possess remarkably well-defined structures that have been applied as model vehicles for drug delivery or template synthesis through attachment of specific ligands with geometrically arranged coat proteins.10 Even in an extremely complex intracellular environment, high-fidelity encapsidation of the genome into a complete virion in vivo is reliably successful. This indicates specific interactions between coat proteins and the genomic nucleic acid, revealing great potential for the controlled formation of viral moieties with DNA scaffold through well-organized protein-protein and protein-DNA/RNA interactions.11

As a proof-of-concept, we employ tobacco mosaic virus (TMV) to realize in situ assembly of viral proteins onto DNA origami to form chimeric nanoarchitectures, in which the sequence-complementarity tethers RNA strands at selected positions to finely tune the assembly the TMV protein.

As illustrated in Figure 1, we first employed a 24-helix bundle (24HB) DNA origami (see Supporting Information for detailed DNA sequences and structures), 16 nm in width and 100 nm in length, as a folding platform that provided addressable toeholds for the in situ assembly procedure. The TMV genome-mimicking RNA strands containing a characteristic loop of the origin of assembly sequence (OAS)12 were readily immobilized on the surface of purified 24HB origami using only Watson–Crick base pairing. Subsequently, after the nucleation of OAS motif binding to a double-layer disk composed of 34 identical TMV subunits, the cooperative assembly proceeded upon the coat proteins tightly piled with RNA and arranged helically around a cylindrical pore. This enables the spontaneous growth of a TMV-like nanotube in a defined assembly pattern until packaging of predesigned RNA is complete,13 thus generating DNA origami-protein nanoarchitectures with programmable protein domains.
It is worth noting that compared to common nanoparticles decorated with multiple linker strands for conjugation with the DNA scaffold, long single-stranded RNA templates for in situ assembly affords mono-functionalization at the bottom of formed protein nanotube with precise controllability but also may compromise the hybridization yield for specific positioning. Herein, we rationally designed a pair of 40 nt neighboring capture strands that protrudes out of the 24HB origami surface for anchoring the 3' terminus of a single RNA sequence (Figure 1). This ensures efficiency and accuracy of in situ assembly of the protein nanotube at a designated docking site on the DNA scaffold.

Figure 2a–c shows typical transmission electron microscopy (TEM) images of protein nanotubes assembled in situ on the 24HB DNA origami. Three types of recombinant RNA strands were used: ~720 nt (short-length, SL), ~1230 nt (medium-length, ML), and ~1910 nt (long-length, LL). These all contain the OAS for stoichiometric control of protein subunits, generating protein nanotubes with theoretically predictable lengths of 30, 54, and 86 nm, respectively (Table S1 and Figure S1). As shown in Figure 2d–f, most of the assembled protein nanotubes on 24HB DNA origami were observed in the length range of 25–35, 50–60, and 80–90 nm, correctly corresponding to the three RNA strand lengths. However, longer RNA sequences can lead to greater length distributions likely due to degradation of the RNA, which was also observed in the blank control (Figure S4). Apparently, the 24HB DNA origami used for anchoring the RNA strand did not disrupt the encapsidation procedure. These protein nanotubes were selectively anchored at specific positions of the 24HB as designed, agreeing with the calculated distance of 25 nm between the fixed point and the top edge of the 24HB origami rod (Figure S5). To confirm the direct attachment of the protein complex at the designed locations, the DNA-RNA duplex connecting the protein nanotube with the 24HB origami was observed in TEM images and could also be destroyed with RNase H (Figures S6 and S7). This further excludes the possibility of protein attachment to the DNA origami through nonspecific binding. Additionally, the yields of the 24HB origami–protein complex using SL, ML, and LL RNAs decreased from ~76% to ~68% and ~56%, respectively, likely reflecting a stronger electrostatic repulsion between the DNA scaffold and the longer RNA strand or more locally accumulated protein oligomers.
Figure 3. Spatially controlled in situ assembly for constructing DNA origami–protein nanotube hybrids. Schematic models (top) and TEM images (bottom) for each hybrid structure. (a) 1D 24HB DNA origami for scaffolding protein assembly, using combinations of (i) SL/SL, (ii) ML/ML, (iii) LL/LL, (iv) SL/ML, and (v) SL/LL RNAs. (b) 2D triangular DNA origami for scaffolding protein assembly, using (i) SL, (ii) ML, and (iii) LL RNAs and combinations of (iv) SL/LL and (v) SL/ML/LL RNAs. (c) 3D tripod DNA origami for scaffolding protein assembly, using (i) one, (ii) two, and (iii) three ML RNAs anchored at the vertex; using three (iv) SL RNAs, each anchored at the end of an arm; and using the combination of (v) ML and SL RNAs anchored at the vertex and arm ends, respectively. Scale bars are 50 nm in the zoomed-in TEM images and 100 nm in the zoomed-out images. See Figures S11-S25 for additional TEM images.

By tuning the positions of the docking sites for the RNA strands on the DNA origami, we were able to spatially arrange the assembly events and achieve intricate DNA–protein configurations. Figure 3a shows the simultaneous growth of protein nanotubes on the centrosymmetric sites of 24HB origami. The shape deformation was expected because of linker flexibility and the drying process required for TEM imaging. We successfully constructed centrosymmetric and asymmetric protein–DNA origami complexes with protein nanotubes extending out from opposite sides of the 24HB origami rod. Protein domain lengths were well-defined, typically ~30, ~54, and ~86 nm, corresponding to the three types of RNA strands. Conventional covalent conjugation procedures are ineffective at protein assembly and orientation through their non-specific modifications on the protein surface. Our in situ assembly using viral protein-RNA and RNA-DNA interaction robustly facilitates the universal and topological control on constructing various DNA-protein complexes.

We next co-assembled protein nanotubes with DNA origami scaffolds folded into 2D triangle and 3D tripod structures. Taking advantage of the sequence-specific binding region at the 3’ terminus of RNA, we precisely positioned SL, ML, and LL RNAs on each of the three vertices of an equilateral triangle origami. This directed the assembly reaction for the formation of five 2D triangular origami–protein nanotube structures (Figure 3b).

The vertex of a 3D tripod origami, was used to assess the competitive assembly of the protein nanotube cluster by quantitatively controlling the docking site number (from one to three) for anchoring ML RNA. We demonstrated incremental increases in the number of ~54-nm protein nanotubes converging at the vertex (Figure 3c, i, ii, and iii), with a maximum number of three, but with greater shape deformation and a dramatically decreased yield of ~11%. In addition to the repulsive electrostatic force, we speculate that steric hindrance contributed to the low
yield, as RNA secondary structure folding has a Gibbs free energy change of approximately ~334 kcal/mol at 37°C (Figure S28). To construct alternate 3D DNA–protein assemblies, we attached RNA strands, e.g. SL RNA, to the ends of the three arms to direct the formation of ~30-nm short nanotubes (Figure 3c, iv). This was also achieved in combination with the assembly of a ~54-nm protein nanotube located on the vertex (Figure 3c, v). Thus, using user-specified DNA scaffolds, our technique provides a simple and general approach for spatial organization of RNA strands to control TMV assembly behavior. Through this, one can achieve versatile DNA origami–protein hierarchical nanoarchitectures with precisely prescribed configurations.

In summary, by engineering recombinant TMV RNAs on DNA origami to locally drive in situ polarized assembly of capsid proteins, we demonstrated a facile strategy to construct various hierarchical DNA–protein geometries on demand. We achieved precise positioning of protein nanotubes on DNA scaffolds via toeholds to anchor OAS-containing RNA sequences that direct the assembly of TMV capsid proteins into nanotubes. Importantly, varying length RNA strands tailored protein nanotube lengths, thereby enabling refined stoichiometric control in a defined growth direction.

Our strategy successfully harnesses the high-fidelity of viral encapsidation and the programmability of DNA origami to realize multicomponent hierarchical assembly in a facile and predictable manner. A dual-function RNA was engineered to attach itself to the DNA origami scaffold and then direct the viral protein assembly. Similarly, a dual-function RNA has been used for the assembly and monofunctionalization of cowpea chlorotic mottle virus (CCMV), and then the CCMV was patterned onto a gold nanoparticle. In addition, several studies have previously described the RNA-directed assembly of viral proteins including TMV1 and red clover necrotic mosaic virus (RCNMV) onto the surface of a colloidal gold nanoparticle. However, compared to the isotropic gold nanoparticle, DNA origami scaffolds enable more versatility in arrangement of viral assemblies in terms of regioselectivity. We expect that the precise spatial control of multiple TMV moieties afforded by DNA scaffolds expands functionalities. For example, TMV nanotubes helically arranged on a DNA axis or patterned on a DNA array can function as a template to construct unique plasmonic structures for sensing applications after deposition of gold or other noble metals. Our methods can also be applied to other viruses, taking advantage of their distinct viral encapsidation procedures. The highly repetitive coat proteins would be good sites to fuse multiple peptides or proteins, greatly benefitting our system in structural complexity and functional diversity.

ASSOCIATED CONTENT

Supporting Information
Experimental details, more TEM images and DNA sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
qbwang2008@sinano.ac.cn

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