RNA-DNA Hybrid Nanoshape Synthesis by Facile Module Exchange

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ABSTRACT: Preparation of nucleic acid nanostructures has relied predominantly on procedures of additive fabrication in which complex architectures are assembled by concerted self-assembly and sequential addition of building blocks. We had previously established RNA-DNA hybrid nanoshapes with modular architectures that enable multi-step synthetic approaches inspired by organic molecular synthesis where additive and transformative steps are used to prepare complex molecular architectures. We report the establishment of module replacement and strand exchange as synthetic transformations in nucleic acid hybrid nanoshapes which are enabled by minimally destabilizing sequence elements such as a single unpaired overhang nucleotide or a mismatch base pair. Module exchange facilitated by thermodynamic lability triggers adds a powerful transformative approach to the repertoire of additive and transformative synthetic methods for the preparation of complex composite materials.

INTRODUCTION

Nucleic acid nanotechnology seeks to create objects, materials, and devices with structural feature sizes at the nanoscale through the folding and association of DNA or RNA components.[1-4] The unique property of nucleic acids to serve both as a building material and a carrier of information encoded in the nucleotide sequence, allows the design and controlled preparation of complex assemblies endowed with diverse functionality. Two fundamentally distinct fabrication methods for nucleic acid nano-architectures include the origami and Lego approaches. Controlled folding of long nucleic acid single strands by hybridization of short staple oligonucleotides gives rise to large origami structures which emerge from a top-down design strategy.[5] Origami approaches to nucleic acid nano-architectures were originally developed for DNA[6] and later expanded to RNA,[7-9] including the cotranscriptional folding of RNA strands.[10-12] The controlled association of autonomously folding RNA units ("tecto-RNA"),[13-14] which resemble Lego building blocks,[15] has been used as a bottom-up approach to assemble complex nano-architectures. Recently, our group developed a versatile kit of hybrid nanoshapes as representatives for an RNA-DNA hybrid nanotechnology strategy that partitions architectural and functional roles between structurally diverse RNA modules and chemically resilient DNA building blocks (Figure 1a).[16] Inclusion of both RNA and DNA strands allows to fine-tune enzymatic and thermodynamic stability of the resulting nanoarchitectures,[17-19] and enables strand-exchange driven by the thermodynamic stability differences between RNA and DNA homo- and hetero-duplexes.[20] For the selection of RNA and DNA components that self-assemble to desired nano-architectures, we devised a rapid combinatorial screening method.[16,21] Iterative mix-and-match screening applied to combinations of RNA and DNA building blocks led to complex hybrid nano-architectures that assemble in a controlled fashion from multiple unique nucleic acid strands.[22] Systematic variation and screening of component combinations further allowed us to prepare nanoshapes in which DNA strands were successively replaced by RNA, eventually furnishing all-RNA polygons.[22]

Origami, Lego, and hybrid approaches for the preparation of nucleic acid nano-architectures bear the hallmark of additive fabrication in which DNA or RNA components associate to a desired structure in a concerted manner or through sequential addition to stepwise assembled intermediates (Figure 1b). Shortcomings of purely additive fabrication procedures, such as cross-reactivity or undesired assembly of building blocks, have been addressed by careful design of synthesis pathways that capitalize on the ability to program nucleic acid module association through selective hybridization of unique sequences and the control of association kinetics.[23-24] The modularity of nucleic acid nanostructures that assemble from multiple different building blocks motivated us to explore strand and module exchange as a synthetic method to transform or derivatize stable RNA-DNA hybrid nanoshapes (Figure 1b).

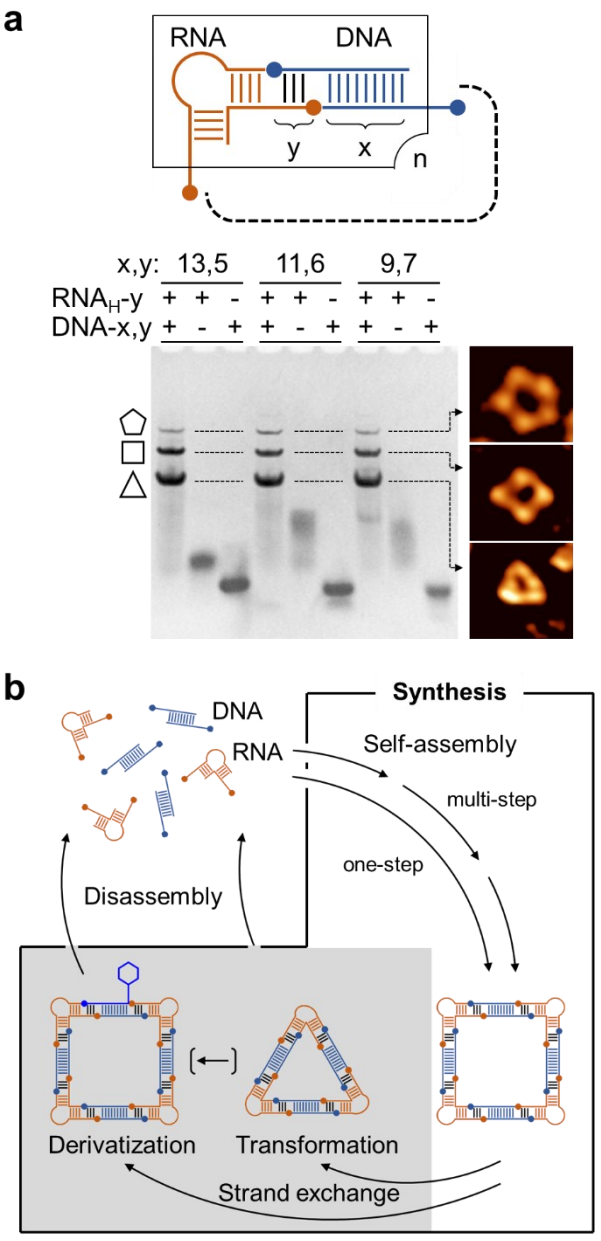

Figure 1. Synthesis of modular RNA-DNA hybrid nanoshapes. (a) Design and preparation of hybrid nanoshapes that contain corner modules derived from an internal loop RNA motif in the genome of hepatitis C virus (RNA$_H$-y) and linear DNA connector modules (DNA-x,y). The number of nucleotides in the complementary hybridization sequences is indicated by y, and x is the number of base pairs in the DNA connectors. We previously described the preparation of stable hybrid nanoshapes incorporating the RNA$_H$-y corner motif separated by a fixed number of 23 base pairs (x+2*y=23) which include the hybridization sequences (y) on both ends of the DNA connectors (x).[16] The thermodynamic stability of nanoshapes increases with increasing length of the complementary hybridization sequences (y). Nanoshapes form as mixtures of polygons which were characterized by native polyacrylamide gel electrophoresis (PAGE) and atomic force microscopy (AFM) imaging.[16] Image inserts with individual nanoshapes are 30 nm wide. (b) Complex nucleic acid hybrid nanoshapes contain RNA and DNA modules that self-assemble through hybridization of complementary sequences. Assembly of nanoshapes may proceed in a single step all-or-nothing process under thermodynamic control, or by multi-step synthesis with intermediates prepared through kinetic or thermodynamic control of component association. Further complexity can be introduced by synthetic procedures of strand exchange that transform nanoshape composition or create chemically diverse derivatives.

By drawing inspiration from organic molecular synthesis, we approached the preparation of nucleic acid nanostructures as a multistep noncovalent transformation that harnesses thermodynamics of self-assembly pathways to sequentially process information stored in the constituent modules towards creation of a desired complex architecture.[16, 22, 24-25] Here, we established strand and module exchange transformations for RNA-DNA hybrid nanoshapes which conceptually correspond to functional group conversion and homologation reactions in organic synthesis. In these reactions, efficient RNA or DNA component exchange in stable nanoshapes was rapidly driven to completion by subtle thermodynamic lability triggers including an unpaired nucleotide and single base pair mismatches. Component exchange in nanoshapes proceeded to substitute individual RNA corner modules or DNA connectors, and to control the shape and distribution of polygon populations. Native polyacrylamide gel electrophoresis (PAGE) and atomic force microscopy (AFM) were used to monitor transformation reactions and to characterize nanoshape products.

RESULTS AND DISCUSSION

Module replacement in RNA-DNA hybrid nanoshapes under thermodynamic control. RNA-DNA hybrid nanoshapes are polygonal nanostructures that self-assemble from RNA corner modules and DNA connectors by hybridization of single-stranded complementary sequences (Figure 1a).[16] While base pair formation between short overhangs of the RNA and DNA building blocks furnishes marginally stable connections, association of the modules in a circularly closed arrangement leads to synergistic stabilization of the resulting nucleic acid architecture. Spontaneous self-assembly of RNA and DNA modules leads to mixtures of hybrid products in which the frequency of triangles, squares, pentagons, etc., is determined by the kinetics of ring closure favouring smaller over larger polygons (Figure 1a). Overall thermodynamic stability of the RNA-DNA hybrid nanoshapes depends on the length of the single-stranded complementary overhangs of the modules. Nanoshapes containing RNA and DNA building blocks that assemble by hybridization of 6-nucleotide overhangs are thermodynamically stable and can be purified and isolated as homogenous samples of a single type of polygon at room temperature. Incubation at a temperature above 30°C leads to slow strand exchange and re-equilibration of purified nanoshapes.[16]

Modularity of the hybrid nanoshape design allows the incorporation of a diverse RNA corner modules and DNA connectors. We previously demonstrated that the connector modules tolerate single stranded extensions at 5' and 3' termini of the constituent DNA strands.[16] Here, we

used strand extensions of 2 nucleotides in the RNA corner modules to serve as short overhangs that facilitate strand invasion for programmed strand replacement of DNA connectors whose association with the RNA modules is labilized at 37°C (Figure 2a). Nanoshapes were formed from RNA corner modules RNA_H-7 carrying single stranded hybridization sequences of 7 nucleotides, and DNA connectors DNA-13,5 with 13 base pairs and 5-nucleotide single stranded overhangs. Association of nucleic acid modules by formation of 5 base pairs between RNA and DNA building blocks furnished stable polygons as shown by PAGE analysis (Figure 2b). Upon addition of DNA connectors DNA-9,7 containing 9 base pairs and 7-nucleotide overhangs, complete replacement of the DNA-13,5 modules by the DNA-9,7 connectors occurred within 30 minutes, driven by the increased stability of the resulting nanoshapes in which base pair formation is maximized in the RNA-DNA module junctions (Figure 2bc). Similarly, DNA-11,6 connectors were efficiently substituted in nanoshapes assembled from DNA-13,5 connectors and RNA corners RNA_H-6 carrying single nucleotide overhangs (Figure S1a). The increase of thermodynamic stability by formation of one additional base pair in each RNA-DNA module junction was sufficient to drive complete exchange of the DNA building blocks.

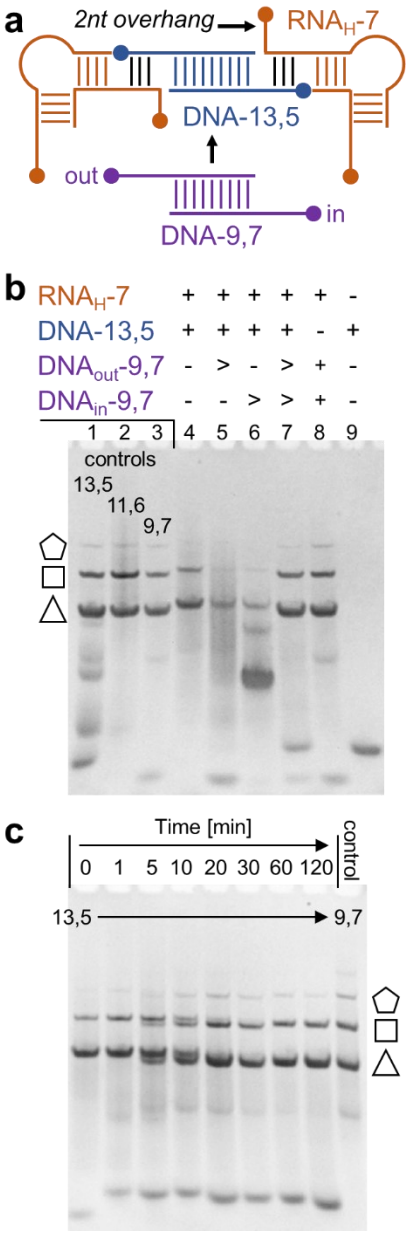

Figure 2. Module replacement in RNA-DNA hybrid nanoshapes driven by thermodynamic stability. (a) Replacement of DNA connector modules in hybrid nanoshapes driven by the thermodynamic stability of products. DNA connectors (DNA-13,5) with 5-nucleotide hybridization sequences associated in nanoshapes with RNA corner modules (RNA_H-7) with 7-nucleotide hybridization sequences (y=7, Figure 1a) can be replaced by DNA connectors with 7-nucleoctide overhangs (DNA-9,7) which increase stability of the resulting nanoshapes by maximizing base pair formation in the junctions between the RNA and DNA building blocks. (b) Native PAGE analysis of combinations containing RNA corner motifs in the presence of both variations of DNA connectors (DNA-13,5 and DNA-9,7). Components present for initial nanoshape formation are marked by +. Addition of DNA modules to pre-formed nanoshapes is indicated by >. Modules with longer complementary sequences of 7 nucleotides (DNA-9,7) replace connectors with shorter sequences in nanoshapes pre-formed with DNA-13,5 (lane 7). Nanoshapes

including the DNA-9,7 modules, which contain 32 nucleotides, migrate faster (lanes 7 and 8) than the polygons with the DNA-13,5 connectors of 36 nucleotides (lane 4). Displaced DNA-13,5 connectors are visible migrating at the bottom of lane 7 (compare with DNA-13,5 control in lane 9). Lanes 5 and 6 are controls to show the impact of adding individual strands of the DNA-9,7 modules. Lanes 1-3 are controls with nanoshapes with fully base-paired components as shown in Figure 1a. (c) Kinetics of module replacement at 37°C by DNA-9,7 connectors in nanoshapes pre-formed from RNA_H-7 and DNA-13,5. At 5 and 10 minutes, both variations of nanoshapes co-exist containing either DNA-13,5 or DNA-9,7 modules. The last lane shows a control with nanoshapes assembled from RNA_H-7 and DNA-9,7 which corresponds to the thermodynamic product of the module replacement time course.

To demonstrate the utility of component exchanges in RNA-DNA hybrid architectures under thermodynamic control for synthetic route design, we explored serial transformation of nanoshapes by successively introducing additional base pairs in the module junctions (Figure S1a). Nanoshape precursors that were initially assembled from DNA-13,5 connectors and RNA_H-7 RNA corner modules, which contained 2-nucleotide overhangs as shown in Figure 2a, were sequentially transformed, first by addition of DNA-11,6 connectors to form nanoshapes with one overhang (Figure S1b, step 1), followed by addition of DNA-9,7 building blocks furnishing the thermodynamically most stable set of RNA-DNA hybrid polygons (Figure S1b, step 2). The sequential nanoshape conversion facilitated by unpaired overhang nucleotides and driven by increasing stability of the RNA-DNA hybrid junctions, which grow in length from 5 to 7 base pairs, demonstrates a general synthetic strategy for the stepwise transformation of complex nucleic acid nano-architectures.

Shape transformation of RNA-DNA hybrid nanoshapes by strand replacement. Homogenous RNA-DNA hybrid assemblies that form a single type of polygon ("homogenous nanoshapes") have been prepared by including a DNA guide strand that carries a defined number of hybridization sites for DNA connector strands.[16] Here, guide strands with 3 or 2 hybridization sites were used to assemble homogenous populations of, respectively, nanotriangles or nanosquares which were characterized by PAGE and AFM (Figure 3ab). Due to the inability of a single 2-site guide strand to promote formation of a circularly closed architecture, two copies of this simple guide DNA function as an alternative to previously used 4-site guide DNA in the assembly of nanosquares.[16] The 3-site DNA guide strands were designed to correspond to DNA connectors with 9-13 base pairs and 5-7 single-stranded overhangs for module association (Figure 3a).

The thermodynamic stability increase of RNA-DNA hybrid architectures by extending the module junctions from 5 to 7 base pairs, which was outlined above as a general strategy of nanoshape transformation, was applied here to devise a sequence of successive synthetic steps that convert a mixture of nanoshape polygons (RNA_H-7/DNA-13,5) to homogenous nanotriangles (RNA_H-7/DNA-11,6) and back to a polygon mixture (RNA_H-7/DNA-9,7) (Figure 3c). Each step proceeded by addition of a new DNA connector component that furnished a product of increased thermodynamic stability. Over the course of these transformations, strand exchange effectively controlled the reversible conversion of a nanoshape polygon mixture to a single type of homogenous nanotriangles. To demonstrate utility of successively extending module junctions as a synthetic method for nanoshape synthesis, we devised a sequence of successive strand exchanges that transformed a mixture of nanoshape polygons (RNA_H-7/DNA-13,5) to homogenous nanosquares (RNA_H-7/DNA-11,6) (Figure S2, step 1) which were further converted to homogenous nanotriangles (RNA_H-7/DNA-9,7) (Figure S2, step 2). This sequence illustrates the use of strand exchange under thermodynamic control as a method to control reversible shape transformation of RNA-DNA hybrid architectures.

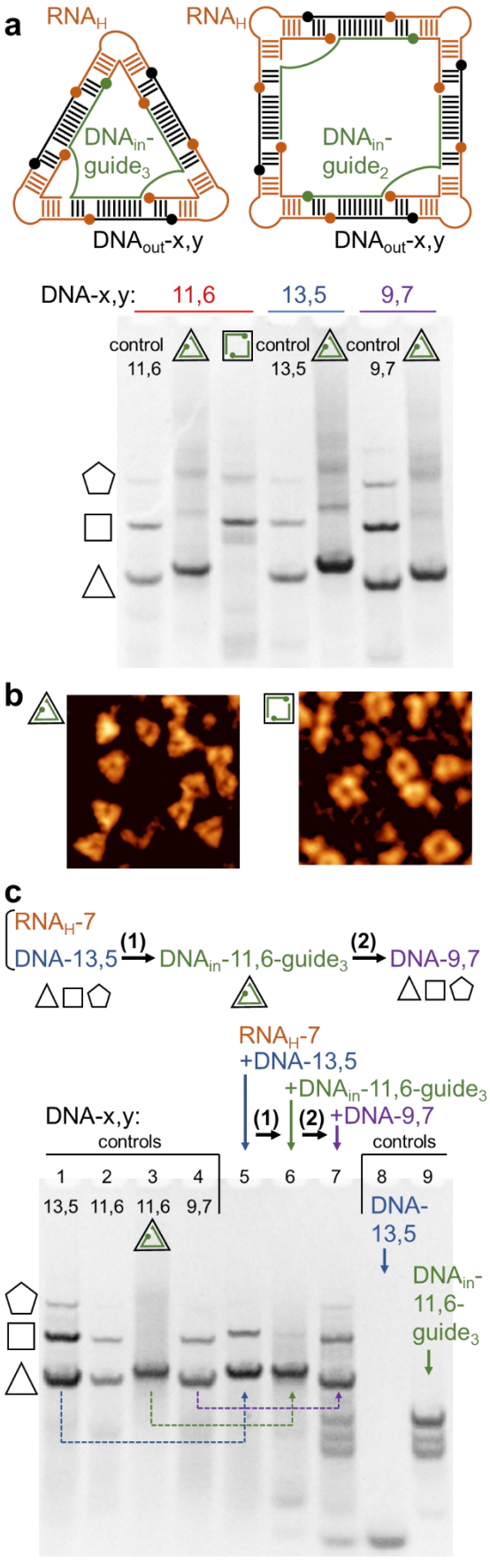

Figure 3. Controlled assembly and strand-replacement transformation of homogenous RNA-DNA hybrid nanoshapes. (a) DNA guide strands with 3 or 2 binding sites for complementary DNA$_{out}$ connector strands direct the formation of exclusively triangles (DNA$_{in}$-guide$_3$) or squares (DNA$_{in}$-guide$_2$) under kinetic control.[16] Native PAGE analysis of homogenous hybrid nanoshapes formed from RNAH corner modules with different DNA guide strands and length of hybridization sequences (y in Figure 1a). Assembly of closed polygon nanoshapes proceeds faster than formation of extended oligomer complexes as attested by the absence of high molecular mass aggregates in PAGE analysis. Faint bands with lower mobility indicate the presence of discrete species at low concentration. While these bands may correspond to larger polygons in which DNA guide strands are partially used, AFM imaging did not reveal such structures likely due to their low concentration. (b) AFM imaging of previously established homogenous hybrid nanoshapes assembled from RNA$_H$-6 corner modules, DNA guide strand and DNA-11,6 connector strands. Image scale is 100 nm.[16] (c) Serial transformation of hybrid nanoshapes from mixtures of polygons to homogenous species and back to mixtures under thermodynamic control at 37°C. RNA$_H$-7 corner modules and DNA-13,5 connectors (Figure 2a) assemble to a mixture of polygons (lane 5) which are transformed to homogenous triangles through addition of DNA$_{in}$-11,6-guide$_3$ strand (lane 6) driven by extension of the module hybridization region from 5 to 6 base pairs. Subsequently added DNA-9,7 modules displace the DNA guide connector and furnish again a mixture of polygons with a more stable hybridization region of 7 base pairs (lane 7). Displaced DNA-13,5 and DNA$_{in}$-11,6-guide$_3$ strands are visible migrating below the polygon products in lane 7 (compare with DNA-13,5 connector and DNA$_{in}$-11,6-guide$_3$ controls in lanes 8 and 9). Lanes 1-4 show control mixtures of polygons with various DNA connectors and homogenous triangles formed with DNA$_{in}$-11,6-guide$_3$ strand (lane 3).

Replacement of RNA and DNA modules driven by resolving a single mismatch base pair. After establishing a synthetic method for the transformation of RNA-DNA hybrid nanoshapes by successive extension of module junctions facilitated by unpaired overhang nucleotides, we explored the utility of mismatch base pairs as a minimal handle for module replacement. To investigate the substitution of RNA building blocks, we first established nanoshapes that contain a different RNA corner module, RNA$_S$-6, which is derived from an RNA domain in the genome of Seneca Valley virus and distinct from the previously used RNA$_H$-6 which was first identified as an RNA motif in hepatitis C virus.[16,26] Module optimization and screening of DNA connectors by PAGE furnished RNA$_S$-6 and DNA-11,6 building blocks that associate to polygon mixtures of stable nanoshapes which were isomorphous to the assemblies formed by RNA$_H$-6 (Figure S3).

To demonstrate RNA module replacement, we prepared hybrid nanoshapes from the RNA$_S$-6 corners and DNA-11,6 connectors which associated through a 6-nucleotide hybridization sequence including a single mismatch base pair in one strand of the RNA corner (Figure 4a). The nanoshapes were incubated with fluorescent Cy3 dye-conjugated RNA$_H$-6 modules that carried fully complementary single-stranded overhangs to resolve the mismatch by Watson-Crick base pair formation. The

fluorescent label served both to facilitate visualization of module exchange and to demonstrate proof of concept for strand replacement as as synthetic method for the preparation of chemically modifed nanoshapes. Complete substitution of RNA_S-6 corner modules was achieved as attested by clean incorporation of the fluorescently labelled RNA_H-6 building blocks to furnish the thermodynamically more stable nanoshapes. In a reversed control of the experiment replacing RNA_S-6 corner modules with Cy3 dye-conjugated RNA_H-6 building blocks, we prepared nanoshapes from fluorescently labeled RNA_H-6 with a mismatch junction and demonstrated facile substitution by unlabeled RNA_S-6 corners with fully complementary overhang sequences (Figure 4b).

Finally, we established that resolution of single mismatch base pairs can be used as a synthetic approach to replace DNA connectors in hybrid nanoshapes (Figure 4c). Nanoshapes were assembled from Cy3 dye-labeled RNA_H-6 corner modules and DNA-11,6 connectors which had a single mismatch in one of the DNA strands. Incubation with TAMRA dye-conjugated DNA-11,6 carrying complementary 6-nucleotide overhangs resulted in incorporation of the fluorescent connectors by displacement of unlabeled DNA. The experiments of DNA connector and RNA corner substitution driven by the resolution of single mismatch base pairs demonstrate that module exchange transformations can be designed to capitalize on subtle differences in thermodynamic stability of hybrid nanoshapes as a method for the chemical modifiaction and synthesis of complex nano-architectures.

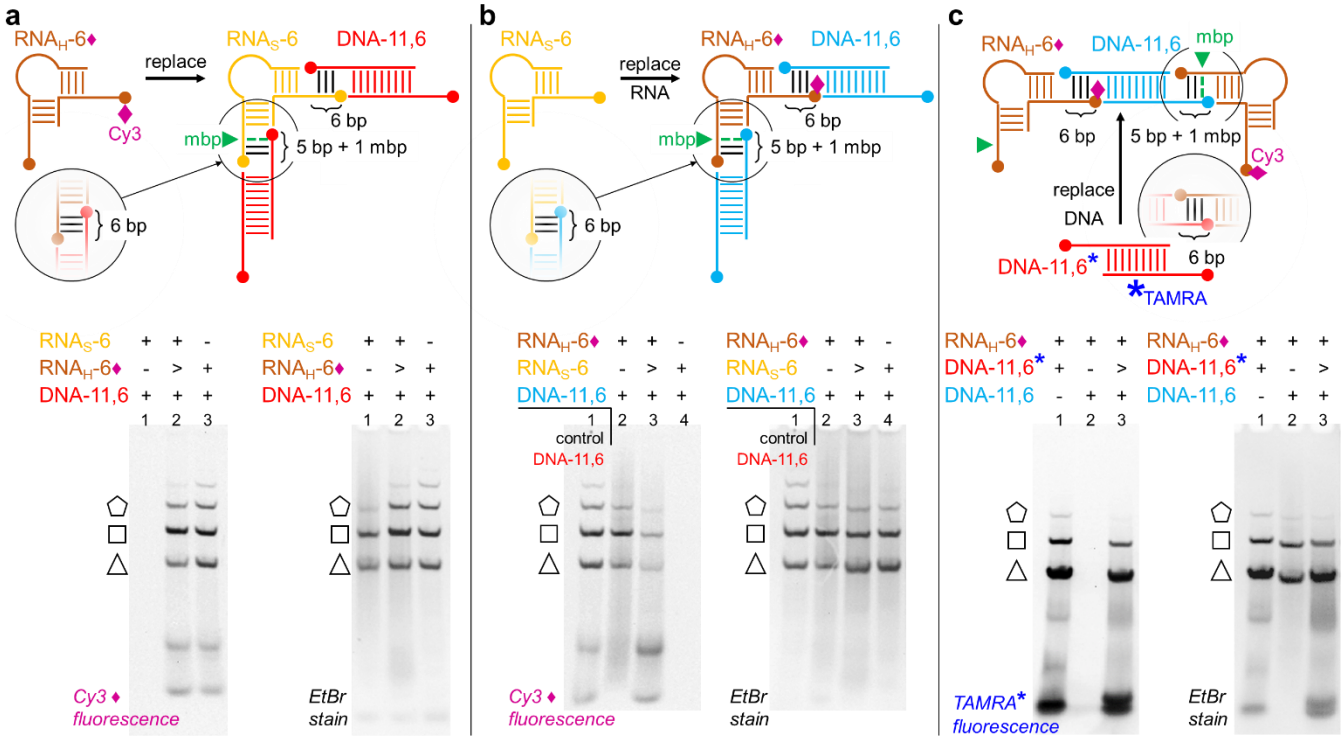

Figure 4. Module replacement in RNA-DNA hybrid nanoshapes under thermodynamic control driven by a single base pair mismatch. (a) RNA_H-6 corner modules (orange) conjugated with Cy3 fluorescent dye can replace RNA_S-6 modules (yellow, derived from an internal loop RNA motif in the genome of Seneca Valley virus) in pre-formed nanoshapes that have a single mismatch base pair (mbp) at one hybridization sequence for association between the RNA corner and DNA-11,6 modules. Corner replacement with RNA_H-6 modules produces thermodynamically more stable connections with 6 base pairs compared to connections with 5 base pairs and 1 mismatch in nanoshapes containing RNA_S-6. Native PAGE analysis confirms the corner module replacement, attested by incorporation of fluorescent dye-labeled RNA_H-6 in nanoshapes pre-formed with RNA_S-6 (lane 2). Lanes 1 and 3 show, respectively, non-fluorescent RNA_S-6/DNA-11,6 nanoshapes and fluorescent RNA_H-6/DNA-11,6 nanoshapes separately prepared as a control. Image on the left was recorded under UV light prior to gel staining to reveal Cy3 fluorescence. Image on the right was recorded of the same gel after staining with ethidium bromide to show all bands containing nucleic acid. (b) Reversed control of the experiment shown in panel a. RNA_S-6 modules (yellow) can replace Cy3-conjugated RNA_H-6 corner modules (orange) in pre-formed nanoshapes that have a single mismatch base pair (mbp) at one hybridization sequence for association between the RNA corner and DNA-11,6 modules. Displacement of RNA_H-6 is demonstrated by largely diminished Cy3 signal in nanoshape bands after addition of RNA_S-6 (lane 3). (c) Replacement of DNA connector module in pre-formed nanoshapes that have a base mismatch at one hybridization sequence between the RNA and DNA modules. Exchange of DNA-11,6 modules was monitored by tetramethylrhodamine (TAMRA) fluorescent dye conjugation in the module with a fully complementary hybridization sequence. Fluorescent TAMRA signal was observed in nanoshape bands after addition of dye-conjugated DNA-11,6 (lane 3), and displaced DNA-11,6 with a single base mismatch in the hybridization sequence appeared migrating at the bottom of lane 3. Replacement reactions were performed at 37°C.

CONCLUSIONS

The modular architecture of RNA-DNA hybrid nanoshapes enables the synthesis and modification of complex nanostructures through multistep noncovalent transformations that capitalize on thermodynamic[16] or kinetic[24] control of assembly pathways to sequentially process information stored in the nucleic acid components. We have previously established systematic combinatorial screening of RNA corner modules and DNA connectors to identify optimal combinations of building blocks that furnish stable RNA-DNA and all-RNA nanoshapes via one-step self-assembly.[16,22,25] Here, we demonstrated proof of concept how labilizing sequence elements, including single-stranded overhang nucleotides and mismatch base pairs, can be introduced as trigger devices for the stepwise transformation of RNA-DNA hybrid nanoshapes under thermodynamic control. Module replacement was established as a synthetic method to modify the building block composition and polygon population of hybrid nanoshapes. Similar trigger device strategies were equally applicable to the exchange of RNA and DNA modules. While we did not investigate strand exchange in all-RNA nanoshapes, our previous studies on the assembly of such nanoarchitectures from RNA corner and RNA connector building blocks suggest that module exchange would be similarly applicable with suitable constructs.[22] We further established synthetic pathways for the preparation of chemically modified nanoshapes by module exchange and demonstrated proof of concept with fluorescent dye-conjugated building blocks. Module exchange facilitated by thermodynamic lability triggers adds a powerful transformative approach to the repertoire of additive and transformative synthetic methods for the preparation of complex composite materials.

ASSOCIATED CONTENT

Supporting Information available containing description of materials, experimental methods, Supplementary Figures S1–S3, and Supplementary Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

S.C. performed experiments. T.H. wrote the paper. All authors have given approval to the final version of the manuscript.

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Notes

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ABBREVIATIONS

AFM, atomic force microscopy; cryo-EM; cryo-electron microscopy; DNA, deoxyribonucleic acid; PAGE, polyacrylamide gel electrophoresis; RNA, ribonucleic acid.

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