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Rational Engineering of Dynamic DNA Systems**

Udo Feldkamp and Christof M. Niemeyer**

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During the past quarter of a century the application of DNA molecules as a scaffold material for constructions in the nanometer range has developed from the design of simple, yet ground-breaking four-armed double-helical junction motifs[1] to the self-assembly of complex structural motifs which serve as building blocks to form large supramolecular constructs.[2] The key property of DNA that makes it so attractive for bottom-up nanotechnology is the extraordinary specificity of the hybridization of single-stranded nucleic acids with their Watson–Crick complements to form stable double helices. Thus, a set of carefully designed oligonucleotides can be programmed to self-assemble into a broad range of diverse superstructures. In addition, reversible transition mechanisms between various stable or metastable states, such as secondary structure conformations, can be engineered into DNA superstructures such that static scaffolds become dynamic nanodevices.[3] Such devices may perform, for example, mechanical work,[4] translate information,[5] aggregate and dissociate nanoparticles,[6] or bind and release proteins.[7] While the design of static DNA arrays nowadays increasingly follows systematic rules which allow the retrosynthetic analysis of desired superstructures to obtain suitable sets of oligonucleotides that will form these structures,[8,9] dynamic DNA devices are usually prepared by individual approaches, which take advantage of various conformational states, transition reactions, and respective triggering stimuli, such as oligonucleotide displacement, changes in ionic strengths or pH values of buffers, or binding of small molecules.[3,10]

In a recent publication, Pierce and co-workes introduced a methodology for the systematic development of dynamic DNA systems which is based on elementary building blocks comprising single-stranded sequences, duplexes, and hairpin loops, and involves hybridization/dissociation pathways that allow various structural conformations to be interconnected.[11] In an initial demonstration of their concept, they describe cascades of hybridization reactions that are programmed to facilitate the step-wise self-assembly of complex static superstructures as well as to accomplish transition between different conformational states of a dynamic DNA device.

The so-called strand-displacement technique is one of the most frequently utilized structural transitions for constructing DNA devices, and this technique has also been used by Yin et al.[11] As shown in Figure 1, hairpin loop structures (red) were designed which contained a short overhang of unpaired bases. This so-called "toehold" can render the hairpin structures metastable.[12] They are stable in their hairpin conformation either alone or in the presence of additional similarly designed hairpin loop species. However, the addition of a so-called displacement oligomer—a DNA strand which contains the perfect Watson–Crick complement of the toehold and the following stem sequence (green in Figure 1)—immediately leads to hybridization and thus, initiation of the branch-migration process. The displacement oligomer completely resolves the intramolecular base pairing of the hairpin strand, thus opening the loop structure—since the resulting long duplex molecule is energetically more

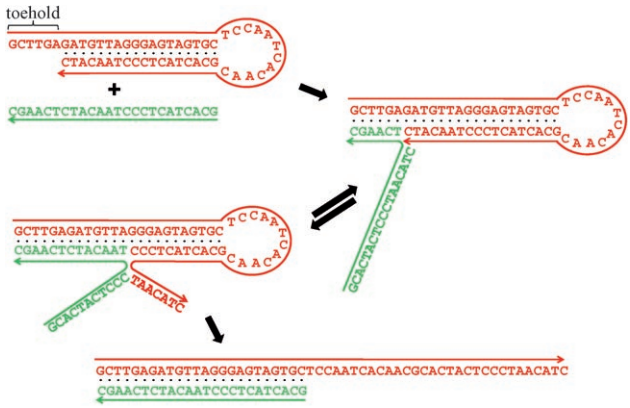

Figure 1. The elementary strand-displacement reaction. The colored arrows indicate the backbones of DNA strands in the 5′ to 3′ direction. The fuel strand (green) hybridizes to the unpaired toehold of the metastable hairpin molecule (red) to form a branched structure. The branching point randomly migrates up- and downstream, eventually replacing the red stem and opening the hairpin. The resulting duplex contains an unpaired end which can undergo subsequent hybridization reactions.

[*] Dr. U. Feldkamp, Prof. Dr. C. M. Niemeyer
Technische Universität Dortmund
Fakultät Chemie
Biologisch-Chemische Mikrostrukturtechnik
Otto-Hahn Strasse 6, 44227 Dortmund (Germany)
Fax: (+49) 231-755-7082
E-mail: christof.niemeyer@tu-dortmund.de
udo.feldkamp@tu-dortmund.de

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favored than the initial hairpin loop containing the short duplex stem. Thus, the displacement oligomer provides "the fuel for strand replacement" and it is, therefore, often called the "fuel strand". The opening of the loop structure changes the role of the hairpin molecule from being the "target" of strand displacement to being a proactive effector molecule, because the unpaired part of the hairpin is now accessible and can, in turn, serve as a new fuel strand for another strand-displacement reaction with another hairpin target. In this way, sequential reaction chains and even complex networks of strand-displacement processes can be executed. Intelligent design of such a reaction cascade will clearly avoid the use of this process for the simple opening of numerous hairpin loops—Instead, each hybridization of a freshly opened loop to another DNA molecule can be designed as one step within the progressive self-assembly of a growing superstructure, or else, as one transition step between various conformational states of a dynamic DNA device.

Pierce and co-workers examplify the flexibility of their method in four quite different applications, which proceed from the rather abstract overall design to the in vitro characterization of the systems.[11] The first example concerns the programming of biomolecular self-assembly pathways and demonstrates the catalytic self-assembly of a three-arm junction. This is a rather simple structural motif which consists of three helical arms joined at a central branching point such that each of the three DNA strands is part of two different arms (see the final product in Figure 2a). Initially, the three strands (**A**, **B**, **C** in Figure 2a) are present in solution as metastable hairpin monomers, each of which contains a toehold. The addition of the initiator molecule **I**, which is

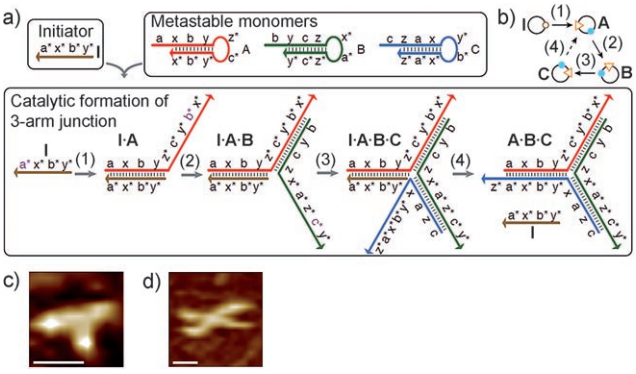

Figure 2. a) Reaction pathway for the self-assembly of a three-arm junction. Through strand displacement, initiator molecule **I** opens hairpin **A**, and then the dangling end of **A** opens hairpin **B** and forms the first arm. Two additional strand-displacement reactions add the other two arms and finally release the catalyst **I**. b) Reaction graph for self-assembly of the junction. The circles (nodes) represent the molecules **I**, **A**, **B**, and **C** shown in Figure 2a, the arrows (edges) represent the intended assembly reactions, and the symbols on the circles represent hybridization domains. Here, the empty triangles indicate accessible toeholds as input domains (see text) and the filled circles indicate the initially inaccessible sub-sequence complementary to the toehold of the next molecule (output domain). c, d) AFM images of the three- and four-armed junction motif, respectively, generated by means of the programmed biomolecular self-assembly pathways. The image is adapted with kind permission from Ref. [11].

complementary to the toehold of strand **A** and its trailing stem sequence, opens hairpin **A** by strand displacement of the complementary stem sequence, labeled y*b*x* in Figure 2a (read from the 5′ end to the 3′ end of the oligomer). The resulting unpaired domain z*c*y*b* is complementary to toehold b and stem sequence ycz of hairpin **B**, thus facilitating the opening of **B** by strand displacement. The resulting product duplex bycz/z*c*y*b* builds up the first arm of the desired three-way junction. The dangling part x*a*z*c* of **B** now attaches to the toehold c of hairpin **C** and opens this metastable structure, thereby producing the second arm. Finally, part a*x*b*y* of **C** hybridizes with ybxa of **A**, thereby forming the third arm of the junction. The last process also releases molecule **I**, which catalytically induces the assembly of additional junction motifs. The successful formation of the three-way junction products was proven by gel electrophoresis and atomic force microscopy (AFM, Figure 2c). The authors then demonstrated that the self-assembly pathway can easily be extended by the involvement of additional metastable hairpin molecules, thus enabling the assembly of higher order junction products, such as a four-armed junction (Figure 2d).[11]

The formation of a dynamic system was chosen as the second example. Here, strand displacement was used for the catalytic formation of DNA duplex molecules (Figure 3). The

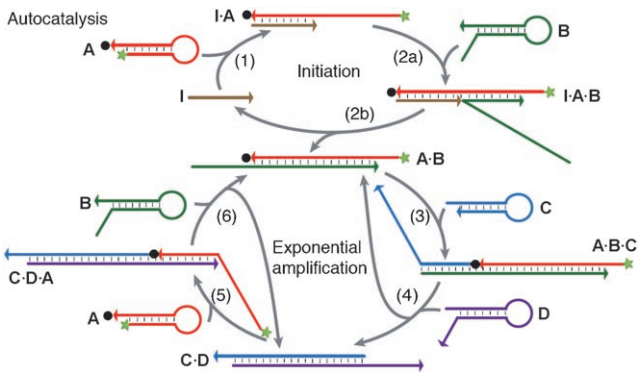

Figure 3. Reaction network for cross-catalytic duplex amplification. In the upper cycle, the initiation molecule **I** catalyzes the formation of the duplex **AB**. In the lower cycle, the unpaired end of **B** catalyzes the formation of duplex **CD**, which in turn catalyzes the formation of further **AB** duplexes (see text for details). The black circle and the green star attached to **A** indicate a quencher and fluorophore, respectively, used to monitor **AB** formation. The image is reproduced with kind permission from Ref. [11].

initiating strand opens a first hairpin structure **A**, which in turn opens another one (**B**). A third strand-displacement reaction then completes the duplex **AB**, thereby releasing the initiator molecule. A sophisticated network of three such duplex-formation cycles was designed to generate a cross-catalytic circuit that exponentially amplifies two duplex species. First, the initiator strand catalyzes the formation of duplex **AB**. An unpaired dangling end of **AB** can now hybridize with the toehold of another hairpin **C** and thus catalytically initiate the formation of duplex **CD**. A dangling end of **CD** is, in turn, complementary to the toehold of **B** and

can therefore initiate duplex formation of **AB**. Thus, the initial hybridization of **AB** triggers a cycle in which not only are further copies of **AB** and **CD** produced, but also each duplex catalyzes the formation of the other duplex species as well, thereby leading to exponential amplification of both duplexes. Similar to the initial work on DNA nanomachines,[4] fluorescence quenching was used for quantitative analysis of the amplification networks, thereby underlining the importance of fluorescence resonance energy transfer (FRET) based spectroscopy for the non-invasive characterization of DNA nanostructure formation.[13]

The third example concerned the self-assembly of yet another scaffold. Here, each opened hairpin hybridized with two (instead of one, as in the previous example) other hairpins. This led to the formation of large dendritic superstructures. Finally, Yin et al. applied their hairpin-opening approach to the engineering of a so-called bipedal "walker". This motif can migrate stepwise along a linear duplex track by hybridizing to and dissociating from hairpins arranged at regular intervals along the track.

The aforementioned examples of strand displacement of well-designed metastable hairpin loops therefore prove the power of this approach to the systematic programming of reaction pathways for the self-assembly of static scaffolds, dynamic devices, and catalytic reaction cascades. As a tool for further systemization of their concept to the engineering of nanotechnological systems, Yin et al. introduced so-called reaction graphs (Figure 2b).[11] In these, a structural motif or conformational state is represented as a node. A symbol is assigned to each node that indicates potential hybridization domains which may trigger conformational changes of either the same (so-called input ports) or of other motifs (output ports). Edges which connect the nodes indicate assembly or disassembly reactions that lead from one motif/state to the other. Such an abstract notation of reaction networks is a helpful tool in the design process, because the functionality and structural transitions of the target devices as well as of intermediates can be fully described using only a few different elements and rather simple rules to compose a graph from these elements. Therefore, the automated translation of a reaction graph into distinct molecules, that is, hairpin motifs and their nucleotide sequences, should be easily achieved.

In summary, the achievement of Pierce and co-workers is (at least) threefold. Firstly, they introduced simple elementary DNA motifs, namely, the metastable hairpin structures, which were used both as building blocks and efficient signal transducers in assembly/disassembly reactions. As impressively demonstrated for several complex reaction pathways, this approach allows for the systematic engineering of static and dynamic DNA nanosystems. Secondly, an abstract formalization of such reaction pathways was developed, thereby moving a large step closer to the fully automated design of DNA-based devices and machinery. Thirdly, the demonstration of functional reaction networks could lead to immediate applications in the area of (bio)sensing. As such, the elegant and fascinating cross-catalytic duplex amplification circuit might be utilized as a sensitive detection system for DNA or RNA targets in diagnostics.[14] It can therefore be anticipated that this study[11] will open the door to a large number of further studies and applications in biosensing and nanobiotechnology.

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