Small molecule-triggered assembly of DNA nanoarchitectures†

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The utilization of toehold-containing DNA strands allows for the assembly of complex nanostructures via kinetically driven hybridization reactions. Here, we have rendered this strategy ligand-dependent, resulting in small-molecule-inducible DNA nanoarchitectures.

DNA nanotechnology is usually described as a bottom-up approach that makes use of the highly predictable design based on specific interactions of Watson-Crick base pairs.^{1–3} The use of DNA as a nanomaterial allows for the assembly of complex nanostructures^{4–7} and scaffolds.^{8–10} Molecular devices such as nanomachines,^{11,12} sensors,¹³ and logic gates^{14,15} have been realized. In addition to the programmable interactions of nucleic acids via hybridization, aptamers possess specific ligand-binding affinities.¹⁶ Although aptamers have frequently been used in biomedical applications,¹⁶ so far only few examples are known where such sequences have been employed to control the formation of complex nucleic acid-based architectures.¹⁷ Here we introduce a general strategy to render kinetically driven assemblies of complex DNA nano-objects ligand-dependent. The approach is based on the small-molecule-triggered liberation of an initiator strand that consecutively catalyzes the compilation of higher-order structures. The approach allows for the assembly of functional DNA nanostructures upon a chemical or biological stimulus.

Recently, Pierce and co-workers introduced an elegant system to trigger catalytic hybridization of DNA strands via the addition of an initiator strand.^{17,18} Hybridization of the initiator *I* to hairpin DNAs (*A*, *B*...) liberates a so-called toehold sequence in the hairpin so that in turn consecutive hybridization reactions can take place. The latter principle of using toehold sequences was first introduced by Turberfield and co-workers, relying on the kinetic control of DNA hybridization.^{19,20} In addition, based on the concept of controlling a conformational change in an aptamer-based system by binding of a small molecule ligand,²¹ a first example of an ATP-triggered assembly of long copolymers formed by two DNA strands has been demonstrated.¹⁷ We wondered whether the triggering of nucleic acid hybridization can be achieved in a modular fashion utilizing other ligands and whether this approach would also allow the formation of more complex DNA nanostructures to be controlled. We have

realized this design for two different ligands by utilizing an ATP²² and an arginine amide²³ aptamer.

For this purpose, we devised an aptamer-locked initiator strand *I* as shown in Fig. 1(a). The aptamer is forced into a

Fig. 1 Ligand-triggered hybridization of nucleic acid strands. (a) Hybridization of the initiator strand *I* to the hairpin *A* is driven via a toehold sequence (red). The toehold is masked by formation of a stem-looped structure (assisted by a blocking sequence, green). Addition of the respective ligand induces folding of the aptamer (blue), liberating the toehold. The liberated 5'-region of the grey strand may contain additional seed regions to mediate hybridization to additional strands as shown in Fig. 2 and 3. (b) ATP-dependent hybridization: native agarose gels showing the ligand-dependent hybridization of the strands *I* and *A*. Reactions containing GTP are shown to prove ligand specificity. (c) Quantification of gels shown in (b): black: ATP; red: GTP. (d) Concentration-dependent kinetics of the ATP-induced hybridization reaction measured by excitation at 495 nm and emission at 520 nm utilizing a FRET-labelled strand *A*, right panel. (e) Temperature-dependency: reactions containing 2 mM ATP were carried out at indicated temperatures and analysed after 10 min (squares) and 180 min (circles).

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misfolded state by formation of a stem-looped structure utilizing a blocking sequence (green) and the toehold (red). Liberation of the toehold (which can subsequently initiate hybridization to a second strand *A*) is triggered upon addition of the aptamer ligand, see Fig. 1.

In Fig. 1(b) ATP-dependent hybridization of strands *I*_{ATP} and hairpin *A* is shown. Initiation of the hybridization reaction is specific for the respective ligand as shown by addition of GTP. For sequences and optimization of the blocking sequence element see ESI Fig. S1.† In Fig. 1(d) time-courses of the hybridization reaction with dependence on increasing concentrations of ATP are shown, indicating that the hybridization reaction takes place within 30 min. The hybridization reactions are kinetically controlled since even in the absence of the ligand hybridization takes place after prolonged incubation times (> 24 h, data not shown). In addition, the reaction rate shows a pronounced temperature-dependence. After 10 min, only 25% of hybridization product is formed at 5 °C as opposed to more than 75% at 44 °C. Importantly, more than 75% product is formed at all temperatures after 3 h, see Fig. 1(e).

Having established the ligand-dependent formation of the hybrid *I-A*, we wondered whether this mechanism could be exploited to trigger a further reaction with an additional strand *B*. We investigated whether the hybridized strand *A* could be used to assemble a duplex *A-B*, see Fig. 2(a). This application has been demonstrated previously but was stimulated upon addition of the initiator strand.¹⁸ In our case, the presence of ATP is necessary in order to efficiently hybridize strands *A* and *B*, see Fig. 2(b). The successful demonstration of the ligand-triggered assembly of the species *A-B* that lacks the aptamer-containing strand *I* should now enable the generation of more complex nanostructures as

Fig. 2 Ligand-triggered formation of a duplex *A-B*. (a) Addition of ATP triggers formation of *A-I* as already shown in Fig. 1. Strand *A* contains a second toehold sequence that is liberated upon binding of *I*, resulting in a consecutive hybridization to *B*. (b) Native PAGE gel showing ligand-dependent formation of the duplex *A-B*, visualized by radioactive labelling of strand *B*. (c) Quantification of gel shown in (b).

demonstrated recently by Pierce and co-workers.¹⁸ We next tested whether higher-order DNA assemblies such as a four-way junction could be assembled from hairpins in a ligand-dependent way, see Fig. 3(a). Indeed, the formation of the anticipated *A-B-C-D* four-way junction was found to be ligand-dependent. For a system triggered by ATP see the ESI.† In principle, the design of the aptamer-containing initiator strand should enable the exchange of the ATP aptamer for other sequences in order to trigger the formation of DNA nanostructures by other ligands as long as the green blocking sequence is adapted to disrupt aptamer folding and to block toehold accessibility. We tested the general approach by utilizing a second DNA aptamer specific for arginine amide.²³ In analogy to the ATP-driven reaction, addition of 25 mM arginine amide pronouncedly enhanced the assembly of the four DNA strands to the four-way junction; see Fig. 3(b) and ESI† for a more detailed characterization of the system. Arginine amide was found to trigger the reaction specifically, since equal concentrations of lysine were unable to induce the hybridization reaction (data not shown).

The presented approach allows for the ligand-dependent assembly of DNA nanostructures which are based on kinetically controlled, toehold-mediated hybridization reactions. Since various interesting applications such as multi-way-junctions, branched structures, autocatalytic hybridization systems, as well as molecular motion have been demonstrated utilizing the

Fig. 3 Arginine amide-dependent formation of a DNA four-way junction. (a) Arginine amide-dependent liberation of *I* triggers the formation of a four-way junction composed of four hairpins *A-D*. (b) Time dependence of the arginine amide-triggered assembly reaction. Progress of the hybridization reaction was assayed via native agarose gels ranging from 15 to 180 min. For details see ESI.†

toehold-based assembly,^{17,18} rendering the formation of functional DNA nanoarchitectures ligand-dependent opens up a variety of applications. Given the huge potential of aptamers due to the diverse nature of the respective ligands, DNA nano-objects should be accessible to be triggered by a variety of chemical or biological stimuli. The transfer of the presented concept into triggering conformational changes in RNA hairpins should allow linking of the formation of functional nucleic acid structures to controlling biological processes such as antisense and RNA interference technologies.^{24–26} In principle, such *in trans* systems could complement recent endeavors in synthetic biology to control specific RNA species^{27–29} via ligand-dependent activation of an initiator strand.

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