DNA Conjugation

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Polyamide Struts for DNA Architectures**

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Owing to its well-established synthetic access, its well-known structural properties, and its size, DNA is a very interesting material for building nanoarchitectures in a bottom-up approach.[1] This has already been proven in a number of studies. For example, cubes,[1a] octahedra,[2a] Borromean rings,[2b] as well as tubes[2c] (nanowires) have been built with DNA. However, DNA can not only be used as a structural material on a nanometer scale but also as a functional material, for example, to measure temperature[3a] force.[3b] However, as DNA is a linear polymer, it is difficult to build stable, branched three-dimensional architectures.[1a] Branched DNA duplexes offer a possible solution, but complex "tiles" have to be used for stable structures.[4a] Other approaches, for example, rely on the use of synthetic DNA derivatives to build trisoligonucleotidyl junctions.[4b]

Polyamides derived from nonproteinogenic amino acids containing *N*-methlypyrrole, *N*-methylimidazole, or *N*-methylhydroxypyrrole groups can bind in a hairpin motif to the minor groove of double-stranded (ds) DNA.[5] The base sequence to which they can bind selectively and with high affinity can be programmed by the sequence of the respective residues in the two antiparallel strands of the hairpin polyamide. A complete set of pairing rules is available for the straight-forward recognition of the majority of possible DNA sequences. DNA-binding polyamides of this type can be used for a variety of applications, like, for example, gene regulation[6a] or as sequence-selective nuclear stains.[6b] As most of the names for this class of compound are either lengthy or imprecise, we propose the term "Dervan-type polyamide" in honor of P. B. Dervan who made the major contributions in this field.

Herein, we report on the use of Dervan-type polyamides as a possible second orthogonal structural element—besides Watson–Crick base pairing—for DNA-based architectures. Our rationale is that by connecting two hairpin polyamides with a long and flexible linker, we generate "DNA struts" that target their matching binding sites on different DNA duplex strands and can thus function as "sequence-selective glue", which will make it easier to build up complex stable architectures. Only once before has a Dervan polyamide been shown to bind across two DNA duplexes, but in this case, the two duplexes were preorganized next to each other in the nucleosome core particle.[7]

To test whether Dervan polyamides alone can be used to hold DNA objects together, we synthesized the heterobifunctional strut **S-AB** shown in Figure 1. It consists of the two Dervan-type polyamides **PA-A** and **PA-B**, which are connected with an ethylene glycol linker. For details on the synthesis and characterization, see the Supporting Information.

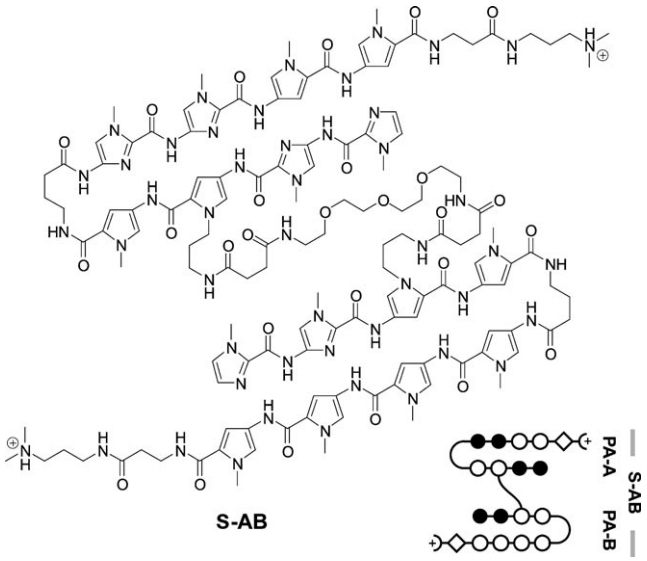

Figure 1. The heterobifunctional strut **S-AB** containing the two DNA-binding subdomains **PA-A** and **PA-B**.

To test our hypothesis of strut-mediated association of DNA duplexes, we used the hairpin DNA molecules shown in Figure 2. The hairpins **H-A** and **H-B** have match binding sites for **PA-A** and **PA-B**, respectively. Each of these binding sites is a double mismatch binding site for the respective other

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

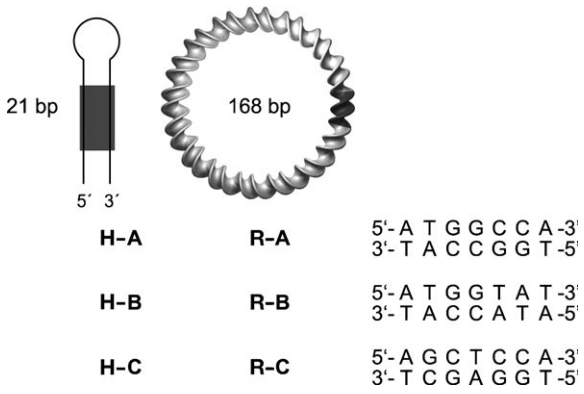

Figure 2. Overview of the DNA hairpins and DNA rings used for the interaction studies. The locations of the binding sites are indicated in dark gray and their sequences are shown on the right.

Table 1: Dissociation constants (K_D) of DNA complexes determined with FCS.

Cy5[a]	RG[b]	Unlabeled	K_D [nM]	Remark
H-A	**PA-A**	–	5 ± 1	match
H-A	**PA-B**	–	n.d.	mismatch
H-B	**PA-A**	–	n.d.	mismatch
H-B	**PA-B**	–	2 ± 1	match
H-C	**PA-A**	–	n.d.	mismatch
H-C	**PA-B**	–	n.d.	mismatch
H-A	**H-B**	**S-AB**	20 ± 5	match
H-C	**H-B**	**S-AB**	n.d.	mismatch
H-A	**H-B**	–	n.d.	
R-A	**R-B**	**S-AB**	30 ± 5	match
R-C	**R-B**	**S-AB**	n.d.	mismatch
R-A	**R-B**	–	n.d.	

[a] This interaction partner was labeled with Cy5. [b] This interaction partner was labeled with Rhodamine Green. n.d. = not detectable.

polyamide, and **H-C** does not contain any match binding sites for either of the polyamides.

The binding behavior of Dervan-type polyamides to dsDNA is usually determined with "footprinting" methods:[8] Although this well-established protocol is very efficient for determining the binding behavior of a binary polyamide/dsDNA complex, it is not suitable to detect a ternary complex like the one consisting of two DNA duplexes and **S-AB**. Therefore, we decided to use fluorescence correlation spectroscopy (FCS).[9]

In this method, two interaction partners are labeled with different fluorophores. What is actually measured are time-resolved fluorescence fluctuations from a very small volume element (approximately 1 fL), which is restricted by the focal volumes of two lasers that correspond to the two fluorophores. To limit the observed species to only few molecules, concentrations in the nanomolar range are used. The time-resolved fluorescence from each fluorophore is sampled and analyzed separately. The observed intensity fluctuations are processed by a hardware correlator and the resulting auto-correlation and cross-correlation curves are analyzed.

Analysis of the correlation curves yields information on the diffusion coefficient and the concentration of the fluorescent molecules in the solution. In the case of the formation of a complex containing both labeled interaction partners, a new fluorescent species arises that is labeled by two dyes. In this way, the concentration of two individual fluorescent species as well as their doubly labeled reaction product can be determined in parallel without the need of separating the two species.

From the concentration of free and bound partners, the K_D value can be calculated. Details of the analysis method have been reported elsewhere[9c] (for experimental details see the Supporting Information). At first, we determined the dissociation constants of the single Dervan-type polyamides **PA-A** and **PA-B** from the three DNA hairpins with FCS. As summarized in Table 1, **PA-A** shows highly specific binding to **H-A**, which contains the match binding site, and likewise with **PA-B** and its matching **H-B**. All other combinations are at least double mismatch situations and, as anticipated, no interaction could be detected with FCS. The K_D values determined for the binding events were in the low nanomolar range, which is in accordance with values reported for **PA-A** and **PA-B** with linear dsDNA.[10,11]

To qualify for serving as a sequence-selective glue for the construction of complex three-dimensional DNA architectures, the DNA structure needs to be capable of holding two DNA duplexes together. To test this, we prepared a mixture of **S-AB** with fluorophore-labeled **H-A** and **H-B** containing the match binding sites. The expected ternary complex could clearly be detected (Table 1). It is equally important that the sequence selectivity of the respective polyamide domains in **S-AB** is retained because when substituting **H-A** for **H-C**, which has only at least double mismatch sites to either part of **S-AB**, no interaction could be detected. This is just like the negative-control experiment, which is performed in the absence of **S-AB**.

Encouraged by these results, we sought to expand this approach to larger DNA objects. Therefore, we synthesized DNA rings made with 168 base pairs and with a mass of 104 kDa. Each ring was constructed from three synthetic DNA fragments of equal length by using a one-pot protocol that enables straightforward access to various rings. To enable ring closure, we introduced poly-A tracts, which are known to induce curvature in linear DNA[12] (see the Supporting Information). Noncircular ligation products were removed by treatment with an exonuclease. One sequence in the ligation was internally labeled to enable FCS measurements.

Table 1 shows the result of the FCS measurements with the DNA rings and **S-AB**, and Figure 3 shows the obtained time course of the cross-correlation function. Again, these results clearly demonstrate the specific formation of the ternary complex between **R-A**, **R-B**, and **S-AB**, but not with **R-C**, which only contains double mismatch binding sites. Without **S-AB**, **R-A** and **R-B** do not form a complex.

In conclusion, we show herein that the heterobifunctional Dervan polyamide **S-AB** can serve as a high-affinity, sequence-selective glue for the controlled assembly of higher-order DNA architectures. Considering the fact that **S-AB** contributes only to 1% of the total mass of the ternary complex, the affinity with which the two rings are held together by this relatively small molecule is impressive. The

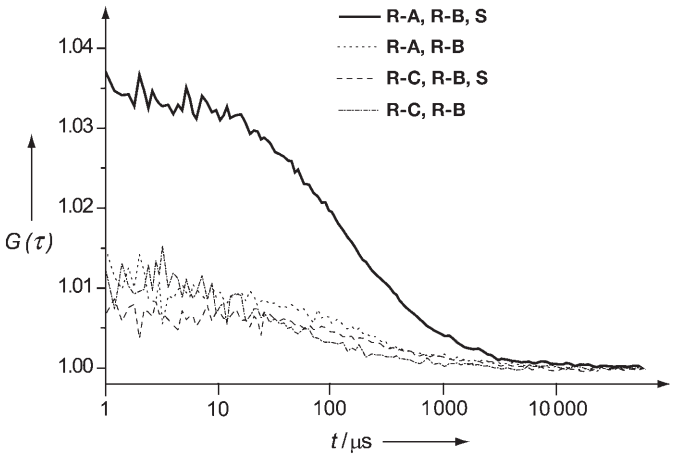

Figure 3. Time course of the cross-correlation function obtained by FCS with the indicated interaction partners.

sequence selectivity is also remarkable as **S-AB** reliably differentiates between two 100-kDa DNA molecules, **R-A** and **R-C**, that differ only in the order of three base pairs. With these properties, the easy assembly of rigid structures made from smaller DNA objects held together by several struts comes within reach.

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