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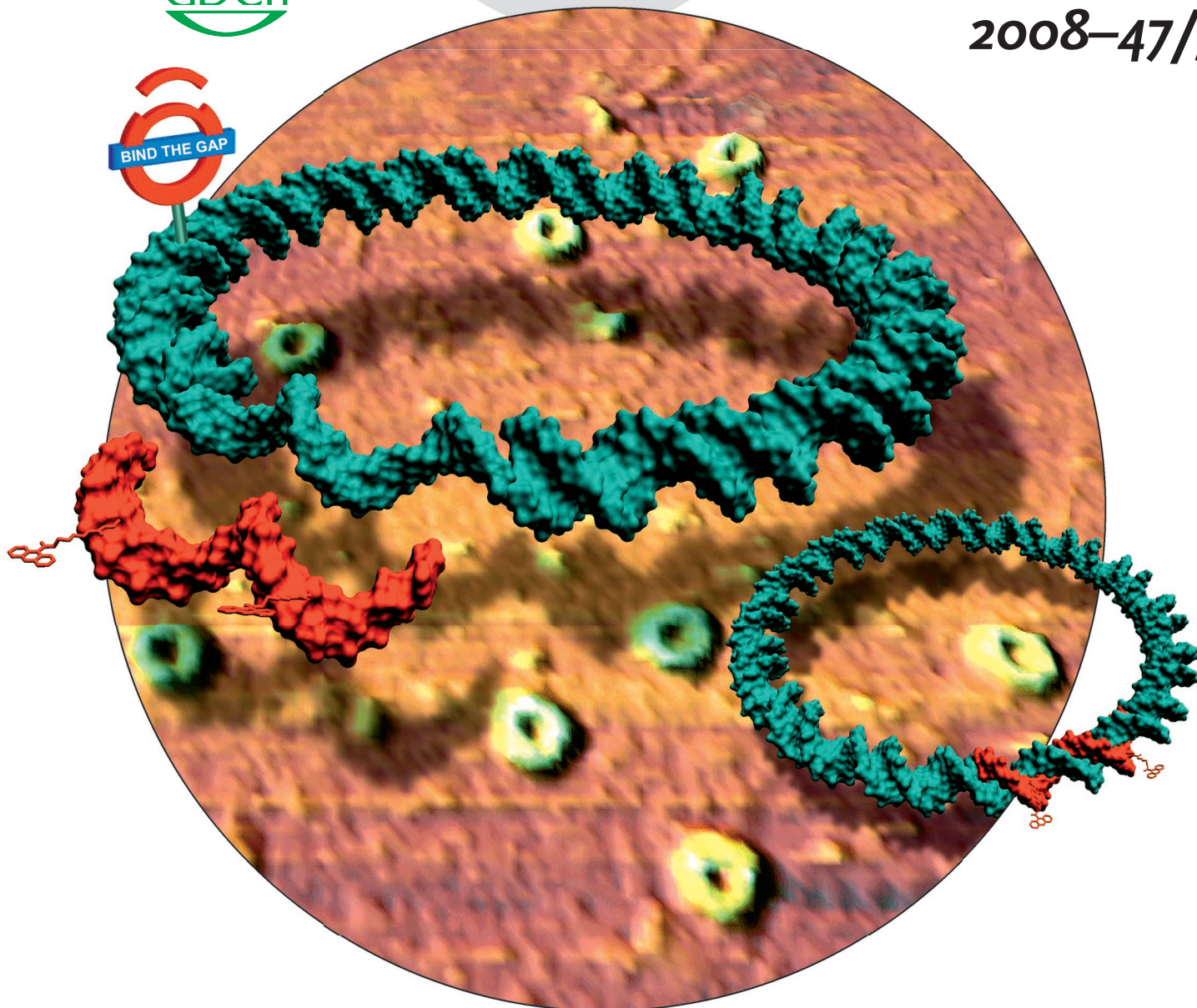
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Olefins in Catalytic Cross-Couplings

T. Rovis and J. B. Johnson

Cleaving Mercury–Alkyl Bonds

H. Strasdeit

Functionalization of P₄

J. M. Lynam

Organic Optoelectronic Materials

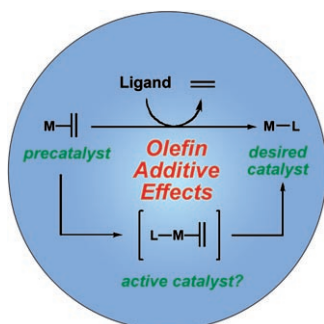
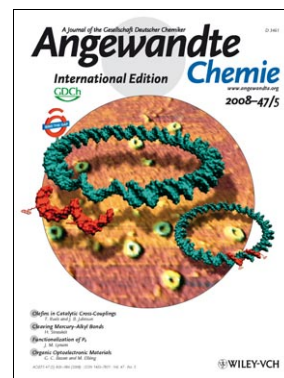
G. C. Bazan and M. Elbing

Cover Picture

Goran Rasched, Damian Ackermann, Thorsten L. Schmidt, Peter Broekmann, Alexander Heckel, Michael Famulok,*

Günter Mayer, Damian Ackermann, Nicole Kuhn, and Michael Famulok*

Bind the gap The cover picture shows an idealized rendering of a DNA minicircle (green) with a 21-nucleotide-long single-stranded region, or gap. M. Famulok and co-workers show in their Communication on page 967 ff. how these circles can be generated and visualized by atomic force microscopy. Any complementary oligonucleotide (red) can hybridize to the gap so that synthetic functional groups can be incorporated. In a second study on page 971 ff. they show that RNA molecules can also hybridize to form a heteroduplex with the gap, enabling the incorporation of nucleic acid motifs in DNA minicircles.

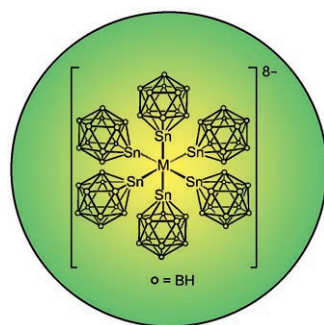
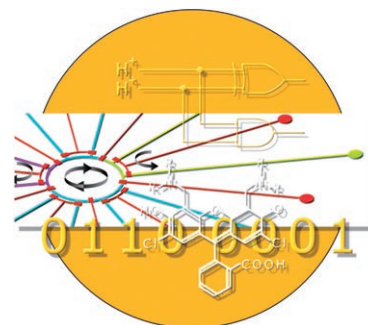


The Influence of Olefins

The activity, stability, or selectivity of catalytic reactions, in particular cross-coupling reactions, can be significantly influenced by olefin components, as T. Rovis and J. B. Johnson show with many examples in their Review on page 840 ff.

Molecular Logic Gates

J. Yoon, S. Park, et al. describe in their Communication on page 872 ff. a programmable microfluidic device based on chemosensors which change their fluorescence intensity in response to various inputs, such as pH value.



Tin Ligands

In their Communication on page 963 ff. L. Wesemann et al. report that in their negatively charged complexes with the tin ligand stanna-closododecaborate, Ni, Pd, and Pt centers have high coordination numbers and high formal oxidation states.

Construction of DNA Architectures with RNA Hairpins**

Günter Mayer, Damian Ackermann, Nicole Kuhn, and Michael Famulok*

There is currently a growing interest in applying nucleic acids as materials for the bottom-up construction of self-assembling nanoarchitectures.^[1] This is mainly due to the ease by which complementary nucleic acid strands can be designed, synthesized, equipped with additional functionality,^[2] and assembled. Consequently, DNA and to a lesser extent RNA^[3] have been used to build a wide variety of geometrical objects,^[4] such as Borromean rings,^[5] tubes,^[6] and two-dimensional shapes or patterns^[7] on a nanometer scale. The vast majority of these structures rely on the powerful self-assembly paradigm of Watson–Crick base pairing, which enables convenient control over the formation of these topological species. However, branched three-dimensional structures or the controlled assembly of different topologies are rather difficult to realize. Possible solutions to this problem are offered by branched DNA or synthetic DNA derivatives, such as trisigonucleotidyl junctions,^[8] or orthogonal struts such as Dervan-type polyamides.^[9] These systems have the possibility to greatly augment the dimensions and complexity of self-assembled nucleic acid based supramolecular systems, by serving as a sequence-specific “glue” that enables the controlled association of multiple functionalized nanoobjects.

Besides Watson–Crick base pairing, natural nucleic acids utilize other structural elements to fold into stable tertiary structures. For example, DNA can form G-quartet structures at the ends of the telomers and in promoter regions which form a multilayer of dG quartets stabilized by central monovalent cations.^[10] Likewise, a plethora of recurring structural motifs of intramolecular RNA–RNA interactions are found in RNA molecules which constitute the modular building blocks of the RNA architecture.^[11] Harnessing these noncanonical natural motifs for the controlled self-assembly of defined nucleic acid based nanoobjects would expand the range of variation considerably.

Here we report the application of short RNA hairpin motifs for the assembly of mixed RNA/DNA architectures. For the basic DNA motif we used 168 base pair (bp) DNA circles (miniplasmids or minicircles), a class of relatively simple but rigid nanoscale objects. For the derivatization of

the circles with RNA hairpin motifs, we used a method which was recently introduced by us and which is based on the hybridization of functionalized RNA oligonucleotides on single-stranded gaps within the otherwise double-stranded DNA circles.^[12]

RNA hairpins with complementary loop regions can interact with high affinity and specificity through the formation of “kissing-loop” complexes that utilize noncanonical interactions. Such kissing complexes occur in natural RNA molecules as regulatory elements, but can also be isolated by *in vitro* selection or obtained by rational design.^[13] We recently identified a short RNA hairpin that interacts with a regulatory RNA domain residing in the 5′-untranslated region of the *thiM* gene of *Escherichia coli* by forming a highly specific kissing complex.^[14] To explore the feasibility of utilizing defined loop–loop interactions for the assembly of nanometer-sized DNA geometries, we isolated minimal versions of these hairpins and added at their 5′ and 3′ ends defined sequences that hybridize with the complementary sequences in defined gap regions of DNA minicircles (circle 1 or circle 2). These sequence extensions allow hybridization with the DNA minicircles through Watson–Crick base pairing (Figure 1A) to form functionalized DNA/RNA minicircles (Figure 1B). As a consequence, the functionalized DNA circles should specifically interact with each other, guided by the RNA hairpins HP1 and HP2, thereby resulting in the formation of kissing circles.

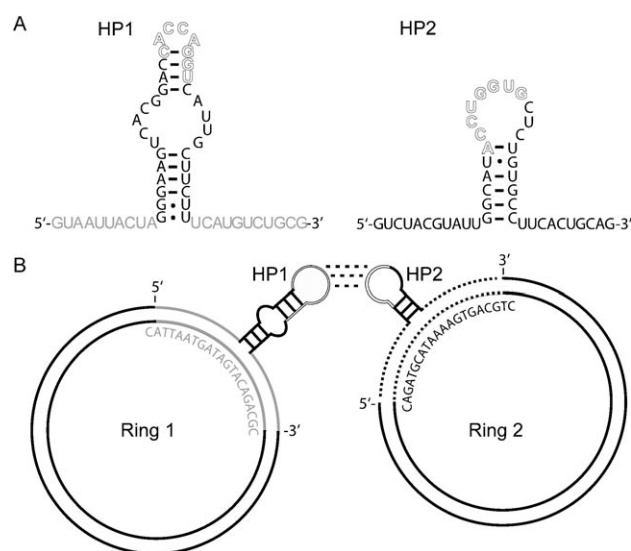


Figure 1. Formation of kissing circles. A) RNA hairpins HP1 and HP2 with adjacent sequences that hybridize to the corresponding gap regions in circles 1 and 2, respectively. B) Functionalized DNA circles 1 and 2 with bound RNA hairpins HP1 and HP2 form kissing circles, guided by the interaction of the RNA hairpins.

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

We first analyzed whether the synthesized RNA hairpins HP1 and HP2 can still interact with one another despite having the adjacent 5'- and 3'-sequence extensions. Therefore, we performed filter-retention analyses using the RNA hairpin HP2 derivatized with biotin at the 5' end and immobilized on streptavidin. These analyses revealed that the hairpins still interact with high affinity, with a dissociation constant of (16.4 ± 3.9) nM in the absence and of (11.0 ± 2.3) nM in the presence of their complementary antisense oligodeoxynucleotides (Table 1 and Supporting Information). These values match with the dissociation constants of the hairpins without the extended sequences.

Table 1: Dissociation constants K_D of the kissing complexes [nM].

Complex	K_D [nM]
HP1-HP2	16.4 ± 3.9
HP1-HP2 + AS ^[a]	11.0 ± 2.3
HP1/circle1-HP2/circle2	8.5 ± 0.6

[a] AS: hybridized complementary oligodeoxyribonucleotide.

Next, we constructed DNA circles with gap regions^[12] complementary to the adjacent 5'- and 3'-sequence extensions of the RNA hairpins HP1 and HP2 (Figure 1 and Supporting Information). The hairpins HP1 and HP2 were hybridized but not ligated to the DNA minicircles. Electrophoretic mobility shift assays were used to visualize the specific hybridization of each RNA hairpin with the cognate circle and also to monitor the formation of the expected quaternary complexes. As shown in Figure 2, the RNA hairpin HP2 specifically interacts with the corresponding DNA circle 2 (Figure 2A, lane 2). No cross-hybridization with DNA circle 1 was observed (Figure 2A, lane 4). As is apparent from the weak, slower migrating band (Figure 2A, lanes 2 and 5), hairpin HP2 also causes a slight cross-linking of two identical DNA circles 2. The reason for this lies in the adjacent sequences of the hairpin which, under the given reaction conditions, interact with two circle equivalents intermolecularly rather than with only one circle equivalent of the same species in an intramolecular hybridization. Thereby, one half of the flanking sequence hybridizes to the corresponding domain of the minicircle, and the other half to the complementary domain of a second circle. However, this interaction exploits only 10 base pairs per minicircle and thus is significantly weaker than the intramolecular hybridization (see the Supporting Information).

Indeed the addition of the second DNA circle/RNA hairpin pair [circle1/HP1] induces the formation of a quaternary complex, which is stabilized by kissing interactions between the loops of the respective hairpin motifs. This is indicated by the strong band with the lowest mobility in the polyacrylamide gel (Figure 2A, lane 6). This band was not observed when only DNA circle 1 or the RNA hairpin HP1 were present (Figure 2A, lanes 3–5). The kissing interactions formed by the loop domains of the RNA hairpin structures require Mg^{2+} ions for the formation of a stable complex.^[13] This situation is illustrated in the crystal structure of the dimerization-initiation site of the genomic RNA of human

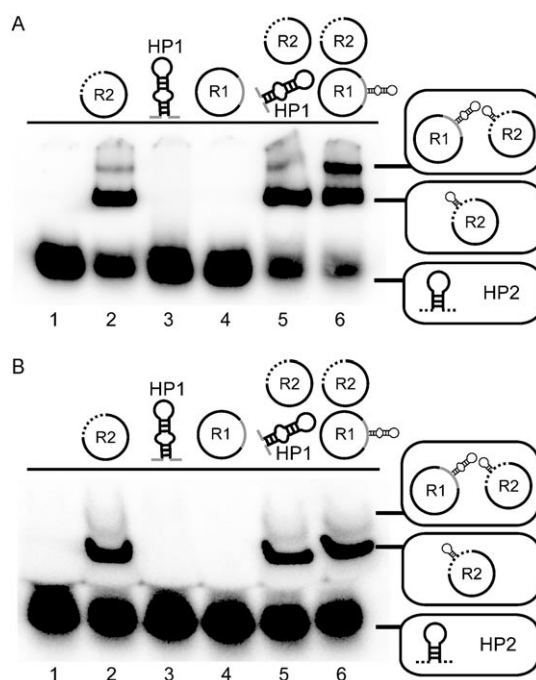


Figure 2. Electrophoretic mobility shift assay showing the specificity of the formation of the kissing circles. A) The radiolabeled RNA hairpin HP2 [30 nM] was incubated either in the absence of RNA hairpin HP1 and circle 1 and 2 (lane 2) or in the presence of HP2 [20 nM] (lanes 3, 5, and 6) as well as the indicated circle 1 or 2 [20 nM] (lanes 2, 4, 5, and 6). B) The same experiment in the absence of the Mg^{2+} ions, which are necessary for the formation of kissing complexes.

immunodeficiency virus type 1 (HIV-1), in which a network of eight Mg^{2+} ions is responsible for the formation of kissing complexes.^[15] Therefore, kissing complexes should not be observed in the absence of Mg^{2+} ions. Exactly this result is evident in Figure 2B, in which the band assigned to the kissing complex (lane 6 in Figure 2A) is not observed in the absence of Mg^{2+} ions, whereas all other interactions that are merely due to hybridization of the interaction partners (which are independent of Mg^{2+} ions) are maintained.

The complexation of DNA minicircles through kissing interactions is concentration dependent (Figure 3). A disso-

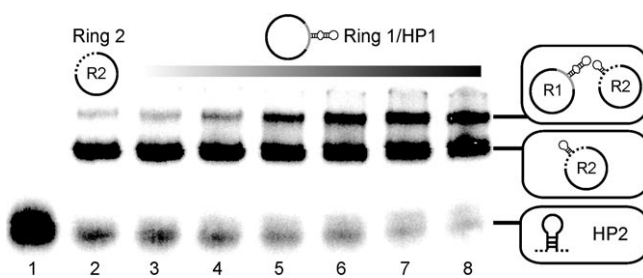


Figure 3. Electrophoretic mobility shift assay showing the concentration-dependent formation of DNA minicircles through kissing interactions. Radiolabeled hairpin HP2 [30 nM] was incubated either in the absence of hairpin HP1 and DNA circle 1 (lane 1) or in the presence of DNA circle 2 [30 nM] (lane 2). HP2/circle 2 complexes [30 nM] were incubated with increasing concentrations of HP1/circle 1 complexes [2.5 nM to 60 nM] (lane 3–8).

ciation constant of (8.5 ± 0.6) nm was determined from the intensities of the bands of the kissing circles shown in Figure 3, (Table 1). This value is in accordance with the dissociation constants observed for the hairpins in the absence of the DNA circles (Table 1).

In conclusion, we have demonstrated here that RNA–DNA chimeras can be used for the construction of nucleic acid architectures. RNA-based loop–loop interactions, so-called kissing complexes, thus represent valuable tools to direct the assembly of DNA molecules. Kissing RNA complexes are found in nature, but in vitro selection protocols allow almost unlimited access to distinct RNA hairpins and probably also DNA motifs that form kissing complexes with defined nucleic acid hairpins. Moreover, kissing complexes reveal remarkable specificity and affinity, as described herein, and therefore qualify as generally applicable, interchangeable, and highly versatile building blocks for the assembly of increasingly complex nucleic acid based architectures.

Experimental Section

Oligoribonucleotides: The RNA hairpins were transcribed in vitro from dsDNA templates generated by PCR using the following oligodeoxynucleotides: HP1: 5'-HP1.F 5'-GATAATACGACTCAC TATAGTAATTACTAGGGAAGTACCGACC-3' and 3'-HP1.R 5'-CGCAGACATGAAAGAAGCAATGACCTGGT-3', template HP1 5'-GGG AAGTCACGGACCACCAGGTCATTGCTTCTT-3'; HP2: 5'-HP2.F 5'-GATAATACGACTCACTATAGTCTACG-TATTGGCATACTGGTGCTC-3' and 3'-HP2.R 5'-CTG CAGT-GAAGGCACAGACACCAGG-3', template HP2 5'-GGCA-TACCTGGTGCTCTGTGCC-3').

Filter-retention analysis: Increasing concentrations of the biotinylated HP2 RNA [1–1000 nm] was incubated with 32 P-end-labeled RNA hairpin HP1 [1 nm] and soluble streptavidin [35 nm] either in the absence or presence of antisense oligodeoxynucleotides [1 μ M] (HP1 AS: 5'-CGCAGACATGATAGTAATTAC, HP2 AS: 5'-CTG CAGTGAAAATACGTAGAC) in binding buffer (10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (Hepes) pH 7.5, 100 mM KCl, 5 mM MgCl₂) for 30 min at room temperature (see the Supporting Information). After incubation, the reactions were passed through nitrocellulose membranes [0.45 μ m] and washed four times with 200 μ L buffer solution. Bound RNA was quantified by Phosphor-Imaging. All assays were performed in quadruplicate.

Electrophoretic mobility shift assays: 32 P-end-labeled RNA hairpins HP2 [20 nm] were incubated with the indicated oligonucleotides in binding buffer at RT for 30 min. After supplementing with 10% glycerol the samples were loaded onto 6% native polyacrylamide gels in binding buffer gels, and run for at least 2–3 h at 4°C (prerun for 1.5 h at 4°C). The gels were then dried and the bands were quantified by Phosphor-Imaging.

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