somewhat universal character, especially in terms of attempts to model amorphous structures exclusively on the basis of the chemical composition. These calculations would inevitably produce the respective energetically most favorable ensembles. In the studied ceramic Si$_3$B$_2$N$_4$ this would imply an alternating occupation of the cation positions by silicon and boron. The need for an incorporation of the genesis of the amorphous network in the theoretical models begins to emerge.

**Experimental Section**

All solid-state NMR experiments were performed on a Bruker DSX400 NMR spectrometer operating at 9.4 T equipped with a 4 mm triple-resonance probe. The resonance frequencies were 128.35 MHz for $^{29}$Si and 79.46 MHz for $^{13}$B. All MAS measurements were performed at room temperature at a rotational frequency of 10 kHz.

$^{29}$Si-isotopically pure Si$_3$B$_2$N$_4$ was prepared from isotopically labeled [2$^{13}$C]trichlorosilylamidochloroborane (TADB). $^{29}$SiCl$_4$, which was obtained by heating a mixture of elemental $^{29}$Si and Pb$_4$Cl$_4$ at 600°C, was allowed to react with hexamethyldisilazane (HMDS) in a ratio of 1:1 to give 1,1,1-trichloro-3,3,3-trimethylsilazane (TTDS). Reaction of TTDS with BCl$_3$ finally led to the target molecule TADB. Condensation of TADB was performed using ammonia. Ammonium chloride, which formed as a by-product, was removed by sublimation at 500°C.

Received: December 27, 1999 [Z14467]

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**Tecto-RNA: One-Dimensional Self-Assembly through Tertiary Interactions**

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The pioneering work of Seeman has demonstrated the use of DNA to construct nanoscale structures.[1–3] Recent work extends these ideas to the assembly of noncovalent complexes, avoiding covalent ligation steps.[4–6] While more chemically labile than DNA, RNA appears to offer a wider range of tertiary motifs[7] that can be used as modular units for supramolecular engineering.[8, 9] “RNA-tectons” refers to the modular character of natural RNA molecules, which can be decomposed and reassembled to create new nanoscale molecular objects. The properties of RNA that facilitate its use in exploring new paradigms in nanoscale chemical self-assembly include (1) ease of sequence-specific synthesis (using either template-driven enzymatic methods or solid-phase chemical methods); (2) amenability of secondary and, increasingly, tertiary structure to rational design,[9] (3) hierarchical folding of individual molecules,[10] and (4) ability to participate in highly specific tertiary interactions.

Here we report the modular design and synthesis of RNA molecules capable of selective dimerization and one-dimensional self-assembly (Scheme 1). In contrast to associations involving Watson–Crick pairings, association occurs by specific tertiary interactions involving hairpin tetraloops and their receptors, as confirmed by lead(ii)-cleavage protection experiments and by motif-swapping experiments.

We chose the specific “11-nucleotide motif” receptor for 5′-GAAA-3′ tetraloops,[11, 12] as the primary unit to mediate specific, high-affinity intermolecular RNA interactions. We first designed the self-dimerizing molecule 1, RL-GAAA (Figure 1). All RNA molecules were synthesized by in vitro transcription of PCR-generated DNA templates (see Supporting Information) using T7 RNA polymerase. The self-association of 1, as monitored by nondenaturing polyacrylamide gel electrophoresis, occurred with submicromolar dissociation constants and definitely required magnesium ions. At 15 mM Mg(OAc)$_2$, 1 dimerized with $K_d = 4.3 ± 0.4$ nM (Figure 2). The binding affinity of 1 was measured as a

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function of $[\text{Mg}^{2+}]$ and the data were fitted to the Hill equation. $K_D(\text{Mg}^{2+})$ was found to be 1 mM and the Hill coefficient was found to be 1.9 ($\approx 2$). In the case of all-or-none binding, the Hill coefficient corresponds to the number of magnesium ions that bind during RNA dimerization. This result is consistent with the observation of one specifically bound Mg$^{2+}$ in the crystal structure of the GAAA tetraloop/receptor interaction.

Motif-swapping experiments and chemical probing with Pb$^{2+}$ were carried out to provide further evidence that dimerization of 1 was, in fact, mediated by specific tertiary interactions and not by complementary Watson–Crick base pairing between two monomers. Motif-swapping has been used to confirm tertiary interactions in natural RNA molecules. First, a single A → U mutation was introduced into the GAAA tetraloop to generate 2, with a GUAA tetraloop (Figure 1). This mutation completely abolished binding even at high magnesium and RNA concentrations (see Supporting Information), most likely by disrupting the stacking of the tetraloop on the “adenosine platform”. In molecule 3 the GAAA-loop receptor was replaced by one for the GUAA tetraloop while the GAAA tetraloop was retained (Figure 1). Molecule 3, like 2, did not self-dimerize. However, 2 and 3 bound to each other with $K_D = 153 \pm 21$ nM. Although the binding is weaker than for 1, it is nonetheless specific and strong enough to allow dimerization at low concentrations of RNA. The weaker binding of 2 and 3 compared to 1 is consistent with previous work on the GUAA loop–receptor interaction.

Computer modeling indicated that, in the homodimer of 1, the GAAA loop of one monomer can be swapped with the receptor of the other monomer. This resulted in a heterodimer comprised of one molecule having two loops (LL in Scheme 1) and a second one having two receptors (RR). Accordingly, molecules 4 and 5, with two GAAA loops and two GAAA-loop receptors respectively, were designed and synthesized (Figure 1). As expected, neither molecule showed any self-affinity. However, they bound to each other with $K_D = 205 \pm 85$ nM. Although the $K_D$ was higher than for the parent 1, it was still in the submicromolar range. Molecule 4 has a flexible single-stranded linker connecting the loop domains and thus lacks the preorganization for binding of the more rigid molecules 1–3 and 5.

Lead(ii)-probing experiments were carried out on 1–5 under conditions favoring either the monomer or the dimer
states (see Supporting Information). Flexible single-stranded regions of RNA are prone to Pb\textsuperscript{III}-induced cleavage, whereas regular A-form helical regions are generally unreactive\textsuperscript{[18]} Moreover, Pb\textsuperscript{III} may substitute at specific magnesium binding sites\textsuperscript{[19]} For 1, Pb\textsuperscript{III}-induced cleavage was observed within the GAAA hairpin and the GAAA receptor motif at concentrations favoring the monomer. These cleavages were dramatically reduced at higher concentrations favoring the dimer. If dimerization were occurring by complementary Watson–Crick basepairing between two molecules, the loop receptor motifs would be regenerated in the dimer and would remain exposed to solvent in the dimer. Thus, the observed Pb\textsuperscript{III} protections (summarized in Figure 1a) are consistent with tertiary binding to form the dimer. Similar results were obtained with the heterodimers, 2/3 and 4/5 (see Supporting Information).

The specific, high-affinity dimerization of molecules 1–5 provided the basis for designing bivalent molecules for linear self-assembly (RRL, in Scheme 1). Bivalent monomer units (tectons) with the potential to self-assemble into one-dimensional arrays were designed by fusing two RL-GAAA molecules at the waist, thus forming the tecto-RNA molecules 6 and 7 (Figure 3). These molecules, synthetized as single RNA transcripts, each comprise a four-way junction with an interaction module on each helical arm. They can adopt two different conformations that differ in the stacking arrangement of the helices at the four-way junction (Scheme 1).

Tectons 6 and 7 were designed to favor one or other of these two conformations by incorporating, in two different ways, the four-way junction sequence from the hairpin ribozyme (Figure 3), for which the stacking preference is known\textsuperscript{[20]} In the energetically more stable conformation of 6, the two helices with GAAA tetraloops (L) are stacked on each other. Thus, tecton 6 is preorganized to self-assemble into a one-dimensional array or to bind 4 or 5, terminating chain propagation in one direction. In the more stable conformation of tecton 7, each helix containing a GAAA receptor (R) is stacked on a helix terminated by a GAAA tetraloop (L). Thus, 7 is preorganized to self-assemble or to interact with 1, which acts as a chain terminator. Both 6 and 7 can potentially isomerize between the two stacking conformations. Thus, tecton 6 can also bind 1 by isomerizing to the stacking conformation of 7. Likewise, 7 can bind 4 or 5 by first isomerizing to the conformation of 6.

Figure 4 shows native gels of the one-dimensional self-assembly of tectons 6 and 7. In the upper panel, increasing amounts of unlabeled 6 were added to titrate-fixed amounts of radioactively labeled 6 or the monovalent molecules 1 or 5. In the lower panel the same experiment was conducted with 7 substituted for 6. These experiments showed that 6 oligomerized very efficiently in the presence of magnesium. As for 1–5, 6 did not assemble in the absence of magnesium. At the highest concentrations of 6 assayed (10 μM), oligomers containing at least 15 molecules of 6 were resolved on native gels. Interestingly, 7 primarily formed dimers. Oligomers of 7 occurred in much lower yield, suggesting that 7 had to undergo a conformational change prior to oligomerization. As expected, 5 bound with higher affinity to 6 than to 7, whereas the opposite was seen for 1. Additional experiments were carried out in which radiolabeled 7 was titrated with 6 over the range 0.02 to 10 μM (see Supporting Information). Increasing the concentration of 6 caused radiolabeled 7 to be incorporated into higher molecular weight assemblies. Concomitant-
long-range goal is the design and assembly of two- and three-dimensional arrays using RNA. Clearly, new rules for RNA self-assembly remain to be elucidated.

Received: January 24, 2000 [Z14592]