Assembly of Mesoscopic Analogues of Nucleic Acids

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The formation of double-stranded DNA by recognition of sequences in single-stranded DNA by complementary sequences is fundamental to life.[1] This type of molecular recognition has been studied at every level of detail and realism, from experimental genetics to theoretical statistical mechanics.[2–9] The work described here introduces an analogue model for sequence-specific recognition, using mm-sized objects (analogues of the bases of DNA, or AnBs) floating at the interface between perfluordecalin (PFD) and water and interacting through capillary forces and shape recognition.[10–12] The immediate objective of this work is to abstract the concept of sequence-specific molecular recognition from biology, and use it to join meso-scale objects. Its longer-term objective is to explore the possibility that the recognition of coded sequences will be useful in precision assembly and in the generation of new types of materials from small components. This work is thus an initial exploration of a new strategy for the fabrication of small, structurally complex, non-molecular structures.

The AnBs, each 3.2 mm in width and ∼1 mm in thickness, were designed to have shape-selective recognition sites (Scheme 1).[13] We used two pairs of AnBs (A·B and C·D) having complementary shapes, in analogy to the A·T and G·C base pairs of DNA; the method could, however, be extended to larger numbers of elements, by analogy to larger numbers with expansions of the four bases used in DNA.[14] The recognition site in each AnB was placed slightly off-center; this asymmetry made sequences of the AnBs asymmetric, and modeled the 5′-to-3′ directionality of DNA. The AnBs were connected by a flexible thread (∼150 μm thick and 400 μm long).

The objects were made from poly(dimethylsiloxane) (PDMS), a hydrophobic, elastomeric polymer (Scheme 1).[10] Each strand was designed as a sequence of AnBs using a CAD program. The design was printed onto a transparency using a high-resolution printer.[15] When strands longer than 25 AnBs were required, each strand had features at each end that allowed the strands to be connected.[16] Using this transparency as a photomask, a base-relief

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(13) The size of the AnBs is large enough to see and manipulate without magnification, and small enough that an interesting number can be incorporated into a single chain and studied in a standard-sized Petri dish.

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(15) The transparencies were created using Freehand 8.0 (Macromedia) as drawing program and printed using a 3386 dpi laser image setter.
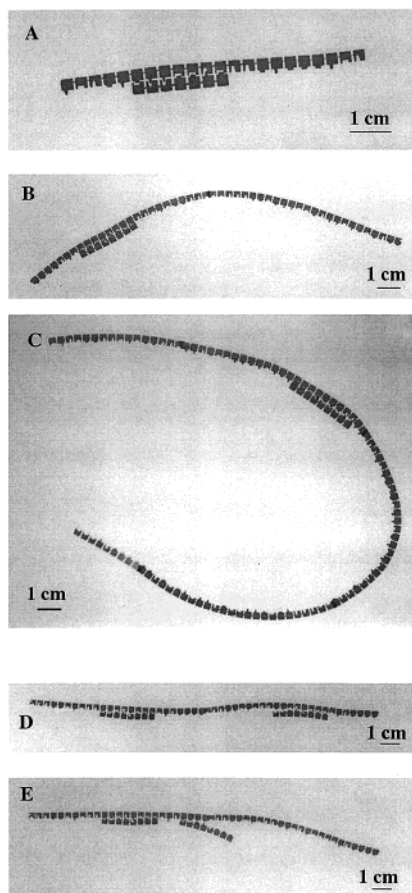
Scheme 1. Schematic Representation of the Model Design and the Procedures Used to Fabricate the PDMS Strands[a]
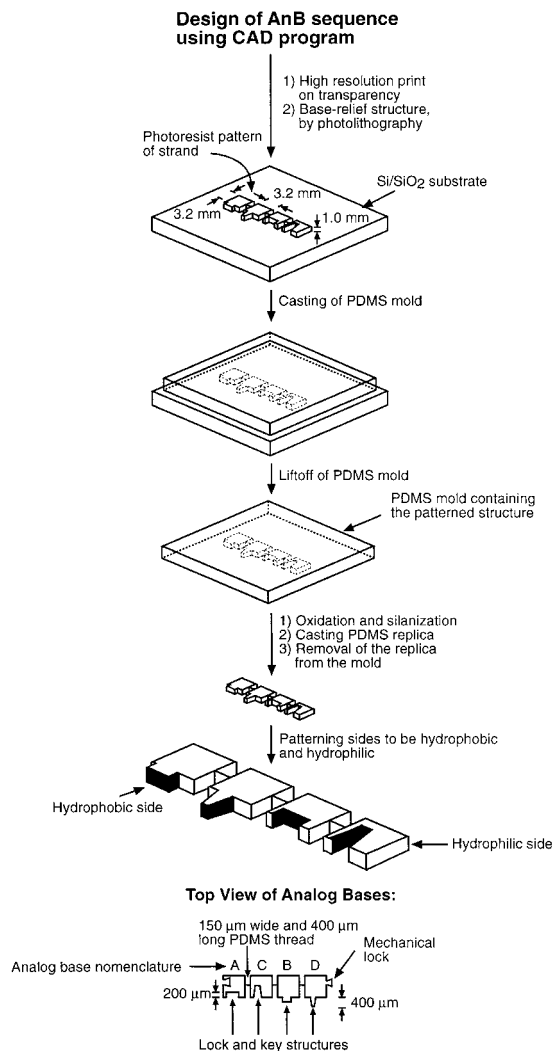
[a] Dimensions are not to scale. The dark faces indicate hydrophobic faces; the light faces indicate hydrophilic faces. In each the top face is hydrophilic and the bottom hydrophobic.

master was fabricated by photolithography.[17] Casting a prepolymer of PDMS against the master generated an elastomeric replica of this structure. This replica served as a mold with which to generate the desired shapes in PDMS. After removal of the strands of AnBs from the mold, they were dyed to make them easily visible. Designated faces of the pieces were made hydrophilic by oxidation in an oxygen plasma.[10] The faces that were to remain hydrophobic were protected before oxidation by covering them either with Scotch tape or with white correction fluid. After oxidation, the protected surfaces were uncovered[18] and the strands suspended at the interface between water and PFD in a Petri dish.[10] The system was placed on an orbital shaker with a radius of gyration of 19 cm and swirled at a frequency of $\omega = 1.0 \text{ s}^{-1}$, using procedures described previously.[10] The two strands paired in 1–3

(16) The 3-in. diameter silicon wafers that we used as substrates for photolithography did not allow fabrication of strands of more than 25 AnBs. Longer strands were fabricated by joining several short strands by hand using the dovetail tongue and groove and gluing.

(17) Xia, Y.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 551.

(18) The correction fluid was removed by carefully taking the pieces off the Scotch tape-covered glass slide with tweezers. The dried correction fluid remained stuck to the Scotch tape; separating the strands from this protective film revealed the hydrophobic faces.

Communications to the Editor

J. Am. Chem. Soc., Vol. 122, No. 14, 2000 3547

Figure 1. Figures show self-assembled structures based on sequence-specific recognition between (A) a 22-membered single strand and a seven-membered probe, (B) a larger single strand (50-membered) and an eight-membered probe, (C) the largest single strand fabricated (100-membered) and a nine-membered probe, (D) a 50-membered strand having two recognition sites and two eight-membered probes. (E) This figure shows the assembly between a 50-membered strand and an eight-membered probe, with one probe correctly paired and the second mispaired.

h (depending on the length of the strand). The double-stranded structures are energetically more stable than dissociated objects, but dissociate when ω is increased.[19,20] We performed each experiment at least 20 times.

A−C of Figure 1 are photographs illustrating recognition of a specific sequence within the long strand by a short probe with complementary sequences. Figure 1A shows the recognition of a strand that had 22 AnBs by a strand that had seven AnBs; association at the correct sequence (sequence: BDDAACA) occurred within minutes. We performed the experiment 20 times: 19 of these times yielded correctly paired sequences; one time the sequence was mismatched. We summarize this result by saying that the probability P of forming the correctly paired sequence was 0.95. We also fabricated longer strands having 50 and 100 AnBs, and allowed them to self-assemble with eight- (sequence: AAADCABB) and nine-membered probe sequences (sequence: CAABBBBCC) (Figure 1B and 1C). For the 50-membered strand, $P = 0.90$; for the 100-membered strand, $P = 0.60$.[21]

The association of the long and probe strands in these processes seemed to be a cooperative process, and occurred several hundred

times faster than the association of single AnBs to the same strand. Interaction of a 50-membered strand with an eight-membered probe that matched no sequence in the longer strand did not result in a stable aggregate.[22]

To extend this concept of site-specific recognition to more complicated self-assembled structures, we investigated the assembly of two short sequences and a long 50-membered strand, modeling the interaction of two probe sequences with DNA. Figure 1D is a photograph illustrating the site-specific recognition of specific sequences within one long 50-membered strand by two short probe sequences. For this double self-assembly, $P = 0.80$. When assembly was incomplete, we observed structures such as that shown in Figure 1E.

These experiments illustrate the self-assembly of mesoscopic systems using a biomimetic process based on molecular recognition of single-stranded DNA by a probe oligonucleotide. The components required for this type of mesoscale self-assembly are easily designed and fabricated. Although this analogue model transforms the complex, three-dimensional molecular interactions of double-stranded DNA into a much simplified form, it retains several key features of molecular DNA recognition: directionality, cooperativity, sequence specificity, and base pairing. There are, of course, significant differences between this mesoscopic model of DNA and molecular DNA: (i) The backbone of the model is both two-dimensional, and more rigid than that of molecular DNA. (ii) The length of the sequence that can be fabricated and probed is very short compared to molecular DNA.[21] (iii) The agitation of the model system is by shear, while agitation in the molecular system is thermal. (iv) The frequency of collision between strands is much lower for the model than for molecular DNA. Although this system is simpler than that of molecular DNA, its behavior suggests that even a highly approximate mesoscopic model of DNA can show behavior similar to that of molecular DNA.[23]

We believe that this system will have several uses.[26] It can provide a physical realization of the models of DNA used in statistical mechanics. It suggests a way to survey uses of DNA in nanofabrication[25,26] or computing[27−31] before carrying out experiments with DNA itself. It may lead to strategies for precision assembly. It provides a simple and easily visualized macroscopic representation of an important microscopic process.

Supporting Information Available: The sequences of all strands (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) We periodically changed the rate of agitation to $\omega = 1.2$ s^{-1} to accelerate collisions, to break up weak structures, and to stimulate annealing.

(20) Self-assembly takes place by the aggregation of one AnB followed by the assembly of the whole probe along the strand. In case of a mismatch, the probe dissociates from the long strand. This process repeats itself several times (on−off cycling) until the correct assembly generates a stable aggregate.

(21) In the case of the 100-membered strands, the dish could only be agitated at a rate of $\omega = 0.9$ s^{-1}. Because the dish had a large diameter, more vigorous agitation resulted in large surface waves and the formation of bubbles of air at the water/PFD interface.

(22) We investigated the self-assembly of small eight-mer probes with two and four mismatches in their sequences: No stable aggregates were observed.

(23) The observation that the probability of pairing is lower in these systems than in molecular DNA can be explained by the different strengths of the agitation, the limitated degrees of freedom of the systems (2D versus 3D) and by the lower probability of collisions between strands.

(24) We believe that this strategy can be applied to AnBs with smaller dimensions; using these structures will make it possible to explore longer and more complex sequences.

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