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Kinetically Controlled Self-Assembly of DNA Oligomers

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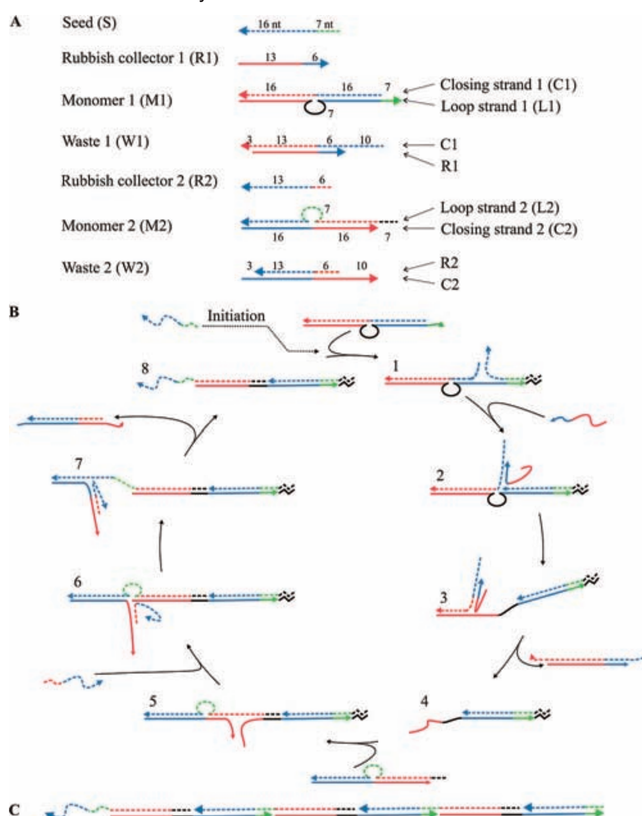
DNA is widely used for programmed molecular construction at the nanometer scale.[1] Assembly protocols usually assume that the equilibrium structure will be formed at each step. DNA has also been used to make machines,[2] including nonequilibrium devices powered by the energy released by DNA hybridization.[3] Nonequilibrium hybridization can also be used to create reaction circuits,[4] and catalysis of the hybridization of DNA hairpins has been used to control the assembly of DNA oligonucleotides into larger structures.[5] Here we present a system for kinetically controlled nonequilibrium self-assembly which employs metastable two-strand monomers[3a] and auxiliary "rubbish collector" strands. This has the advantage that the resulting linear assemblies do not incorporate self-complementary sequence motifs (self-complementarity is characteristic of a hybridization chain reaction based on hairpin loops[5]) allowing flexible sequence design and increasing product stability.

The assembly chain reaction is outlined in Scheme 1. Scheme 1A shows the components: two two-strand monomers (M1, M2), two auxiliary rubbish collectors (R1, R2) and a seed (S). Each monomer consists of a closing strand (C1 or C2) hybridized to complementary domains of a loop strand (L1 or L2) to create two duplex necks, isolating the central domain of the loop strand as a single-stranded (ss) loop and leaving an exposed toehold[6] at one end. The loop domain of L1 is complementary to the toehold of L2 and vice versa, and the neck domains of L1 and L2 are complementary. The designed product of the kinetically controlled assembly process is a linear chain formed by staggered hybridization of many copies of L1 and L2 (Scheme 1C shows a 6-mer). Waste products W1, W2 are formed by hybridization of a closing strand to a rubbish collector strand.

Scheme 1B shows the mechanism. Assembly is initiated by seed S which interacts (1) with M1 by hybridizing to the external toehold on L1. (2) S displaces half of C1 from L1, opening the loop. The last six bases of C1 to be revealed form a toehold to which R1 can hybridize. (3) This allows R1 to displace all but three bases of L1 from C1 by branch migration. Displacement is completed by spontaneous dissociation of W1 (R1+C1) from L1 (4). (5) The opened loop of L1 can now bind the toehold of M2, allowing the newly uncovered neck of L1 to displace the first half of C2 from L2 and (6−7) allowing rubbish collector R2 to remove C2 completely, creating a ss overhang that has the same sequence as S. (1) This overhang can now bind the toehold of a new M1, initiating its incorporation in the growing chain, etc.

Assembly is kinetically controlled. The secondary loop structures of the monomers are kinetically stable.[7] Only the monomer currently attached to the end of the growing chain is reactive because its loop domain is opened,[3a,b,7,8] revealing the toehold required to initiate the next strand displacement reaction. A closing strand is almost completely unreactive while hybridized to a loop strand: reaction with a rubbish collector is enabled only when part of the closing strand is displaced from a monomer by reaction with the growing chain.

Scheme 1. Assembly Chain Reaction [a]



[a] (A) Components of the self-assembling system. Lengths of subsequences in nucleotides are indicated. DNA sequences drawn in the same color but different line style are complementary. ss DNA is indicated as a single line, double-stranded DNA is indicated as a double line. (B) Autonomous cycle of kinetically controlled self-assembly. Zigzag lines indicate the product of previous assembly cycles. (C) The product of non-equilibrium assembly, a linear oligomer consisting of loop strands L1 and L2 hybridized in a staggered configuration with one reactive overhang on the left hand side and the seed incorporated on the right.

Assembly is driven by the energy released when unpaired bases in opened loops hybridize to complementary toeholds.[9] In the metastable initial state each monomer contains 32 bp and 14 unpaired nucleotides, of which 7 are constrained in a bulge loop.[10] Rubbish collectors are ss. When the loop strand of a monomer is incorporated into the growing chain its loop is opened and it forms 23 bp with the free end of the chain. The waste complex contains 19 bp. Overall, incorporation of a monomer leads to the formation of 10 bp with a free energy change $\Delta G° \approx 20$ kcal mol^{-1}.[11]

Hybridization reactions were carried out in 100 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris·HCl and 1 mM EDTA at pH 8.0. DNA concentrations were deduced from measurements of absorbance at 260 nm. The two monomers were prepared separately by heating and rapidly cooling mixtures of loop strands (1 μM) and closing strands (1.25 μM, to ensure that all loop strands are closed). Assembled monomers were mixed stoichiometrically to give final

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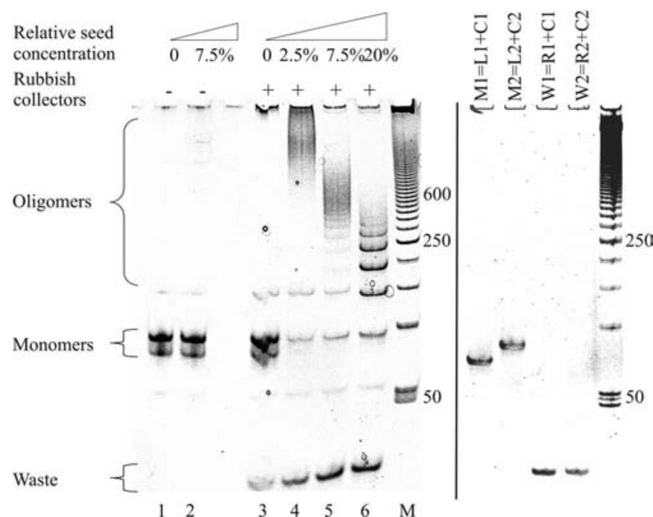

Figure 1. Nondenaturing PAGE (15%) of kinetically controlled DNA self-assembly. Lane 1: monomers. Lane 2: monomers and seed (7.5% of monomer concentration). Lane 3: monomers and rubbish collectors. Lanes 4−6: monomers, rubbish collectors, and seed. Lane M: 50 bp DNA ladder. The gel on the right is a control with the most important strand combinations. For additional combinations see Supporting Information.

loop strand concentrations of 0.5 μM. Rubbish collectors were added to final concentrations of 0.75 μM. Different amounts of seed were then added: oligomerization was allowed to proceed for one hour at 18 °C.

Analysis of the reaction by polyacrylamide gel electrophoresis (PAGE) is shown in Figure 1. Lanes 1 and 2 contain both monomers (lane 2 also contains seed) but no rubbish collectors: products of high molecular weight are almost completely absent, indicating a very low background rate of polymerization. Lane 3 contains monomers and rubbish collectors but no seed: little evidence of assembly is visible, even after 24 h (data not shown), indicating that the monomers are very stable. (Waste complexes in lane 3 result from hybridization between rubbish collectors and excess closing strands.) Upon adding seed (lanes 4 to 6) monomers disappear and a ladder of slower bands appears, showing that oligomers are forming, as designed. There is a broad distribution of product sizes, with an inverse dependence of the average length on seed concentration. At the highest seed concentration (lane 6) the reaction is expected to take on average five steps producing an oligomer containing 115 bp and a 23-nucleotide ss overhang. At 2.5% seed concentration (lane 4) it is expected that the average oligomer contains forty monomers or around 900 bp. Oligomer mobilities are reduced by nicks in the DNA backbone at 23 bp intervals,[12] preventing direct comparison with the DNA marker ladder. Residual monomers in lanes 4−6 indicate a mismatch (<20%) between monomer concentrations. Faint bands in all lanes, running to around 50 bp and 150 bp, are attributed to dimerization of closing and loop strands. Also visible in lanes 4 to 6 are waste complexes.

The presence of the seed at one end of the chain prevents the formation of rings. DNA rings, which form sharp bands with low mobilities,[13] are absent in the kinetically controlled assembly reaction. They are, however, the dominant products when the reactants are annealed to overcome the designed kinetic barriers to reaction (see Supporting Information).

Phosphorylation of one type of loop strand would allow ligation and subsequent separation of a periodic single strand of DNA. This offers practical advantages over assembly PCR[14] (in separation of a single strand) and rolling circle replication[15] (by controlling both 3′ and 5′ terminal sequences) in the synthesis of repetitive ssDNA for use as a nanostructure template.[2h,16]

In conclusion, we have demonstrated the synthesis of linear DNA oligomers by nonequilibrium assembly. The triggered assembly reaction avoids ring structures that dominate when the reactants are annealed. The use of two-strand monomers avoids any requirement for substantial self-complementarity in the oligomeric reaction products. Oligomer lengths can be controlled by adjusting the seed concentration. This assembly system may be of practical use in the synthesis of repetitive linear DNA.

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Supporting Information Available: DNA sequences, additional experimental details, and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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