Model Therapeutic Particle Assemblies with Reversible Linkers

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Statement of Purpose: An ideal therapeutic vehicle often involves delivery of multiple therapeutic and diagnostic agents. The tunable affinity and reversible association between complementary oligonucleotide strands makes DNA a remarkable materials assembly tool; however, past investigations have focused on reversing association between complementary surfaces via thermal denaturation of DNA linkages. Here, our goal is to expand the use of DNA linkages to model, multiparticle therapeutic assemblies in which competitive hybridization is employed at a fixed temperature to release particles from DNA-linked assemblies.

Methods: Multiple copies of amine-terminated DNA were conjugated to polystyrene microspheres. The presence and activity of the immobilized DNA strands on the microspheres was quantified via flow cytometry using FITC-labeled target strands. The target strands contained eight to sixteen base-pair (bp) matches with the probe sequence. Confocal microscopy studies then followed to study DNA-mediated colloidal assembly and disassembly experiments. For these reversible assembly experiments, two sets of particles were conjugated with DNA strands containing 10 bp matches to assemble particles via primary hybridization events. To disassemble the particle aggregates, suspensions were then incubated overnight in a solution of oligonucleotide strands containing 14 bp matches overnight at room temperature. For select cases, 1) a diluent strand (which does not participate in hybridization activity) was added or 2) perfectly complementary strands were replaced with strands containing a single point mutation.

Results/Discussion:
Flow cytometry results indicated that 1) a primary target sequence with 10 bp matches results in significant primary hybridization activity of immobilized probe sequences and; 2) a secondary target sequence with 14 base pair matches results in significant displacement of primary target sequences containing 10 bp matches. Colloidal assembly experiments indicated DNA linkages formed between complementary particles containing 10 bp matches. Particles disassembled following incubation with soluble oligonucleotides containing 14bp matches. The time required for disassembly was reduced for cases involving either 1) immobilized diluent strands in which the number of DNA linkages (via primary hybridization events) was reduced or 2) strands containing a single point mutation in which the duplex affinity (for primary hybridization events) was reduced.

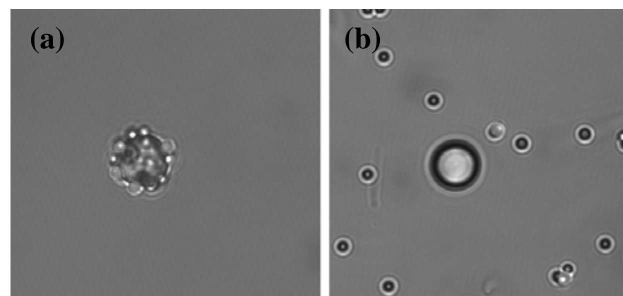

Figure 1. Phase contrast micrographs showing DNA-mediated (a) assembly between particles containing 10 base-pair matches and (b) disassembly of particles incubated in a solution of DNA containing 14 base-pair matches for the large template particle. The assembly consists of one 5μm template or core particle surrounded by complementary 1μm particles.

Conclusions: Collectively, flow cytometry and colloidal assembly experiments indicate that only a four-base difference in the longer, secondary sequence is needed to drive the competitive displacement of soluble primary target strands and cause DNA-linked particle assemblies to break apart at a fixed temperature.