# Design and synthesis of dynamically assembling DNA nanostructures

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Kinetically controlled isothermal growth is fundamental to biological development, but it remains challenging to rationally design molecular systems that self-assemble isothermally into complex geometries via prescribed assembly and disassembly pathways. By exploiting the programmable chemistry of base pairing, sophisticated spatial and temporal control have both been demonstrated in DNA self-assembly, but largely as separate pursuits. This dissertation extends a new approach, called developmental self-assembly, that integrates temporal with spatial control by using a prescriptive molecular program to specify the kinetic pathways by which DNA molecules isothermally self-assemble into well-defined three-dimensional geometries.

First, a new general-use computational sequence design platform, called Multisubjective, is discussed. Multisubjective identifies the specific bases that are responsible for undesired secondary structure, and redesigns only those bases using one of the supported client designers. It combines features of combinatorial and thermodynamic designers, judiciously targeting the thermodynamic analysis to specific candidate designs on an occasional basis and using the fast combinatorial algorithms to do most of the design work. This allows Multisubjective to successfully design large, complex nucleic acid systems in a reasonable amount of time.

Second, the synthesis and characterization of a wireframe DNA tetrahedron is presented as a proof of principle for developmental self-assembly. Nine DNA reactants initially co-exist in a metastable state, but upon catalysis by a DNA initiator molecule, navigate 24 individually characterizable intermediate states via prescribed assembly pathways to arrive at the tetrahedral final product. In contrast to previous work on dynamic DNA nanotechnology, this developmental program coordinates growth of ringed substructures into a three-dimensional wireframe superstructure, taking a step towards the goal of kinetically controlled isothermal growth of complex three-dimensional geometries.

Lastly, design approaches for developmental self-assembly must take into account potential structural problems specific to hairpin structures that can interfere with the kinetics of the desired strand displacement reactions. Preliminary data is presented regarding the effect of tail structure, including toehold–tail interactions, on the hairpin opening kinetics.