

Towards sequence-defined polymers with DNA-based catalytic templating

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Autonomous synthesis of sequence-defined polymers from a pool of monomers is considered to be one of the most important and difficult challenges in modern Chemistry and Synthetic Biology. In contrast, biological systems execute this task with astonishing efficiency while synthesizing nucleic acids and proteins from the available pools of NTPs or aminoacyl-tRNAs. Such accurate biopolymer syntheses are directed by information-bearing templates. In this work, we discuss the design of a DNA strand displacement network in which a catalytic DNA template assembles non-covalent DNA assemblies of length longer than two without the use of biological tools or external physical stimuli.

Forming a longer polymer via any template-assisted method has its own challenges. Firstly, a product consisting of multiple building blocks will have multiple recognition sites to stick to the template. This makes the autonomous detachment of template from the product harder due to increased co-operativity. Second, generating a product more than two units long always has a chance of producing smaller or truncated copies instead of the correct product. Third, the template has to be specific to be able to form the product with correct sequence of the building blocks.

Here we demonstrate the catalytic formation of products of length three to five units using single-stranded DNA templates under non-enzymatic isothermal conditions. We build this system by combining toehold- and handhold-mediated strand displacement reactions (Fig. 1a) that was recently developed in our group¹ along with the insights from a coarse-grained model² of the templating mechanism. First we show that we can make trimeric products (Fig. 1b) using DNA templates that works catalytically with high specificity. We noticed very low production of truncated products in this process. We followed the elementary steps of the network by fluorescence measurements, and validated the product formation by polyacrylamide gel electrophoresis (PAGE) of the aliquots from the reaction mixtures alongside pre-annealed controls. Then we further refined our design to produce tetramers which also showed turnover of the template (Fig. 1c). We finally extended the system to make pentameric assemblies. Our latest results shows definite signs of pentamer formation in presence of the template (Fig. 1d).

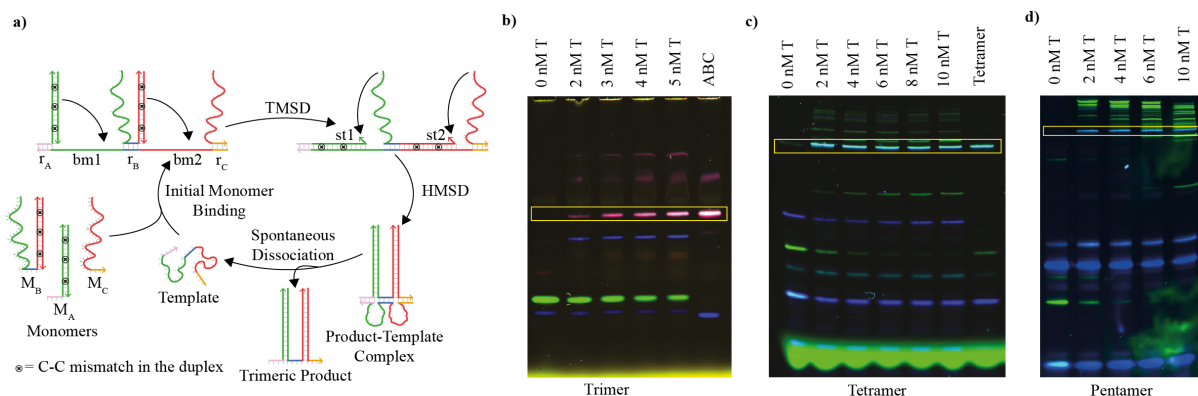


Figure 1: a) Mechanism of catalytic templating to produce a trimer. b-d) Fluorescence image of a polyacrylamide gel alongside pre-annealed controls. Lanes from left to right show the effect of increasing template concentrations added to a mixture of monomers. The yellow rectangle shows the corresponding product bands: b) trimer, c) tetramer, and d) pentamer.

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2. Juritz, J., Poulton, J. M., Ouldrige, T. E. Minimal mechanism for cyclic templating of length-controlled copolymers under isothermal conditions. *J. Chem. Phys.* 156, 074103 (2022).