Characterizing Leakless Strand Displacement Systems

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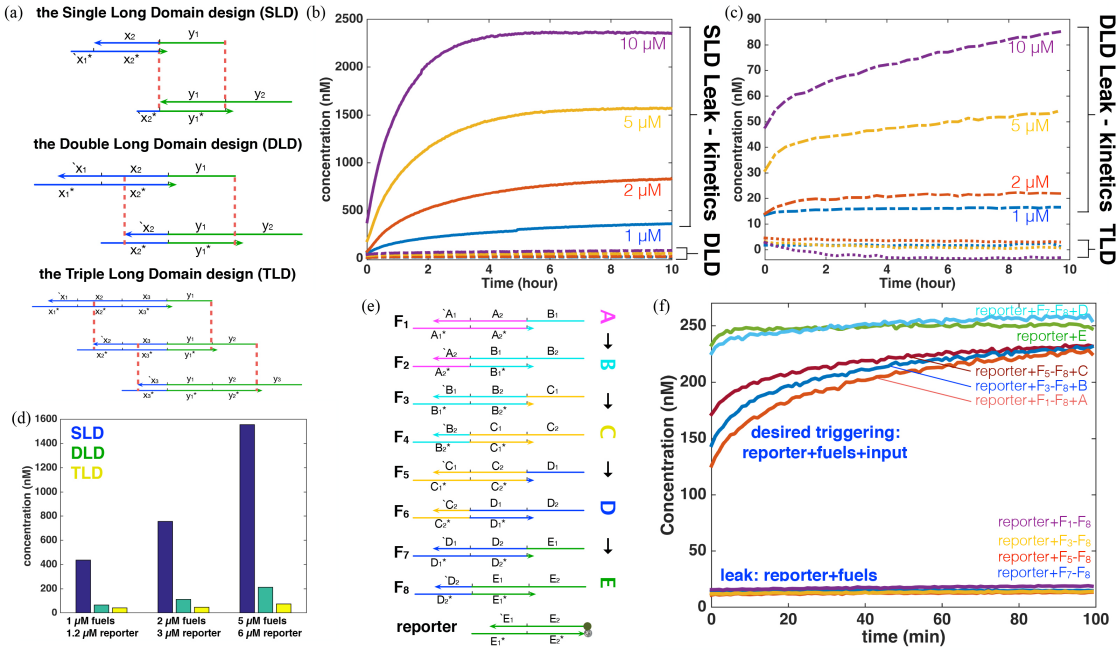
The DNA strand displacement reaction has been extensively used in dynamic DNA nanotechnology. However, DNA strand displacement systems are vulnerable to undesired triggering reactions (leak) in the absence of the correct invader (input) strand. Leak critically impedes the application of strand displacement reactions by reducing sensitivity and disrupting desired system behavior. It also forces strand displacement systems to operate at lower concentrations, and thereby sacrifice speed. We experimentally investigate a domain-level design to reduce system leakage, starting with the basic "translator" primitive that converts a signal of one sequence into that of another. Compared with the traditional single long domain (SLD) design, logical redundancy in the new design ensures a higher energy barrier to leak. Our design strategy is, in principle, capable of reducing leak to arbitrarily low levels, and we experimentally test two levels of leak reduction: double-long domain (DLD), and triple-long domain (TLD). The leak fraction after 10 hours in the DLD design is over 20 times less than the SLD design. We also show that *gradual leak was not measurable* as system redundancy is further increased in the TLD design, even for concentrations as high as 10μM — 100 times the concentration typical of SLD systems. Beyond kinetics, we confirm the theoretical prediction that the amount of leak in the DLD and TLD scheme is lower at thermodynamic equilibrium, which puts an upper bound on the *total* leak. We also implemented a DLD translator cascade with 9 layers of strand displacement reactions. In contrast to the fast triggering in the presence of input (which reaches 1/2-completion before the very first data point), the leak is barely measurable over 100 minutes. Our leakless designs set a possible foundation for future development of fast and robust dynamic DNA systems. This work represents an experimental verification of theoretical results presented at DNA21[1].



(a) The scheme of translators using traditional SLD design, new DLD and TLD design. (b) Kinetics of leak for SLD and DLD in the absence of input at 37℃. (c) Kinetics of leak for DLD and TLD in the absence of input at 37℃. (d) Leak after fuels and reporter are slowly annealed for 18 hours from 90℃ to 25℃. (e) DLD translator cascade that has 4 layers of translators with 8 fuel complexes and a reporter. (f) Kinetics of the translator cascade for desired reaction in the presence of input and leak reaction in the absence of input at 25℃. [reporter] = [fuels] = 500 nM, [input] = 250 nM.

[1] C. Thachuk, E. Winfree, and D. Soloveichik. Leakless DNA strand displacement systems. In DNA Computing and Molecular Programming, pages 133−153. Springer, 2015.