A Simple Colorimetric Assay Using Toehold-Mediated Strand Displacement Triggered Gold Nanoparticles Disassembly

Trang Nguyen, Michael Lam, and Julianne Gibbs Davis*

University of Alberta

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Since the development of DNA functionalized gold nanoparticles in 1996, there has been an enormous growth in the use of gold nanoparticles (AuNPs) in various biomolecular assays. In our work, we take advantage of the unique optical property of gold nanoparticles to design a simple and rapid colorimetric detection platform based on toehold-mediated strand displacement. Toehold-mediated strand displacement has been shown to play an important role in controlling hybridization processes in DNA based assemblies. Herein, we systematically study the effect of toehold length on the stability of a DNA duplex linked AuNP aggregate, where the toehold, or overhang, is present on the linker strand. Next we monitored strand displacement and subsequent aggregate dissolution caused by the introduction of a target strand that was completely complementary to the linker. We found that the melting temperature decreased with increasing overhang length on the linker DNA length due to the steric effects of the toeholds or overhangs. Importantly, when the target strand was made thermodynamically favourable (i.e. by increasing the toehold length), the dissociation of these aggregates worked at any temperature between 25.8-29.8°C, the latter being close to room temperature. This work demonstrates that incorporating a toehold into the experimental design makes our colorimetric strand displacement assay less temperature sensitive than our previous system explored by our group which lacked any toehold and thus only exhibited rapid target triggered strand displacement close to the aggregate melting temperature. Our results indicate that toehold-mediated strand displacement can overcome the thermodynamic difficulties of our strand displacement triggered AuNP disaggregation process.

Schematic of hybridization and dissociation induced by toehold-mediated strand displacement