[**Supporting Information** to accompany Manuscript]

Tuning Toehold Length and Temperature to Achieve Rapid, Colorimetric Detection of DNA from the Disassembly of DNA-Gold Nanoparticle Aggregates

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Table S1. DNA sequences used for hybridization and disassembly of aggregates.

Probe A:	5'- (HS) AAA AAA AAA ATT GTT AAA T-3'
Probe B:	5'- ATT GAT AAG AAA AAA AAA A (SH)-3'
Linker:	5'-CTT ATC AAT ATT TAA CAA-3'
Target:	5'-TTG TTA AAT ATT GAT AAG-3'
Linker + oA_1	5'-A CTT ATC AAT ATT TAA CAA A-3'
Linker + oA_3	5'-AAA CTT ATC AAT ATT TAA CAA AAA-3'
Linker + oA_5	5'-AAAAA CTT ATC AAT ATT TAA CAA AAAAA -3'
Linker + oA_7	5'-AAAAAAA CTT ATC AAT ATT TAA CAA AAAAAAA -3'
Linker + oA_9	5'-AAAAAAAAA CTT ATC AAT ATT TAA CAA AAAAAAAAA -3'
Target + oT_1	5'-T TTG TTA AAT ATT GAT AAG T-3'
Target + oT_3	5'-TTT TTG TTA AAT ATT GAT AAG TTT-3'
Target + oT_5	5'-TTTTT TTG TTA AAT ATT GAT AAG TTTTT-3'
Target + oT_7	5'-TTTTTTT TTG TTA AAT ATT GAT AAG TTTTTTT-3'
Target + oT_9	5'-TTTTTTTTT TTG TTA AAT ATT GAT AAG TTTTTTTTT-3'

Table S2. Melting temperature of free linker and complement target DNA[1]

$oA_1 + oT_1$	T_m = 55.1 °C
$oA_3 + oT_3$	T_m = 60.3 °C
$oA_5 + oT_5$	T_m = 64.2 °C
$oA_7 + oT_7$	T_m = 67.3 °C
$oA_9 + oT_9$	T_m = 69.8 °C

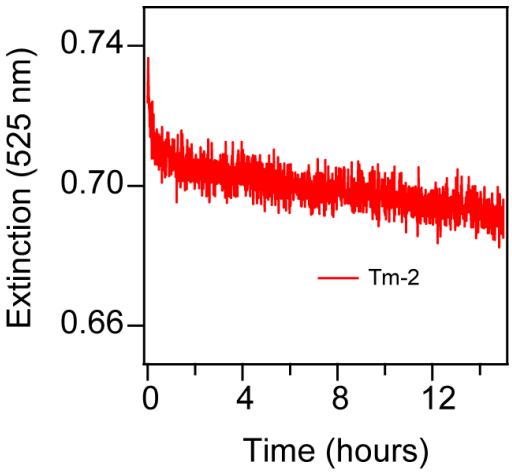

Figure S1: Aggregation of DNA-AuNP with linker DNA at $T_m - 2$ (35.5 °C). The two AuNP probes and linker DNA were combined at time zero. The absorbance change was minimal after 13 hours indicating that hybridization and aggregation was incredibly slow at this temperature. Experimental conditions: 60 pmol of linker DNA and 1.5 pmol of both AuNP probes in 1 mL buffer (0.5 M NaCl, 10 mM PB, 0.01% SDS, pH 7). The samples were stirred at 200 rpm throughout the experiments.

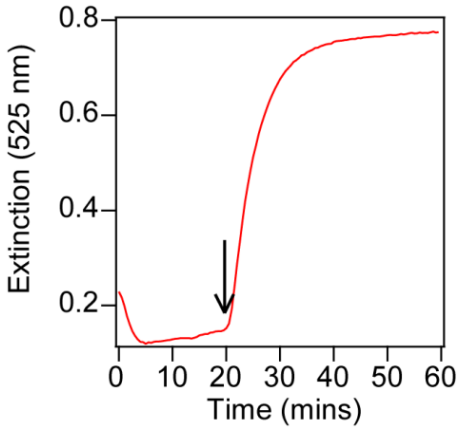

Figure S2: Results of monitoring a target triggered strand displacement at λ = 525 nm. Sample is initially placed in a cuvette set at 35.6 °C ($T_m - 2$) and allowed to equilibrate for 20 minutes before the addition of target (arrow). Even after 20 minutes of equilibration, there is no melting that occurs at this temperature and the disassembly can be attributed to the addition of target. Experimental conditions: 60 pmol of linker DNA and 1.5 pmol of both AuNP probes in 1 mL buffer (0.5 M NaCl, 10 mM PB, 0.01% SDS, pH 7). Target (60 pmol) is added at 20 minutes and the samples were stirred at 200 rpm throughout the experiment.

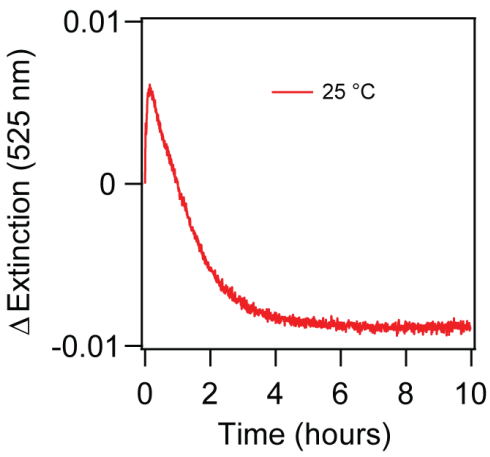

Figure S3: Monitoring the kinetics of target-triggered strand displacement (without toeholds) at 25 °C (Tm - 12 °C). The absorbance change is monitored at 525 nm for 10 hours and shows an initial increase in absorbance upon target addition and a slow decrease over a span of 10 hours. Experimental conditions: 60 pmol of linker DNA and 1.5 pmol of both AuNP probes in 1 mL buffer (0.5 M NaCl, 10 mM PB, 0.01% SDS, pH 7). Target is added at time zero and the samples were stirred at 200 rpm throughout the experiment.

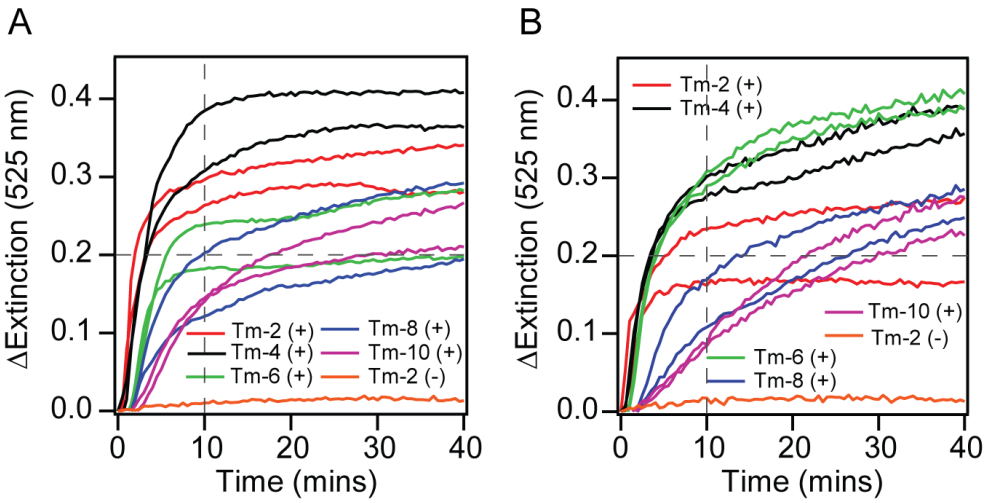

Figure S4: Duplicated experimental results of toehold mediated strand displacement with (A) 7 base overhang and (B) 9 base overhang. Dotted grey lines denote the criteria for selecting successful assay. As overhang length increases the broadness of the melting transition increases therefore resulting in self melting during the equilibration period of 20 minutes at Tm-2. Consequently, the change in extinction is less due to partial melting in both (A) and (B).

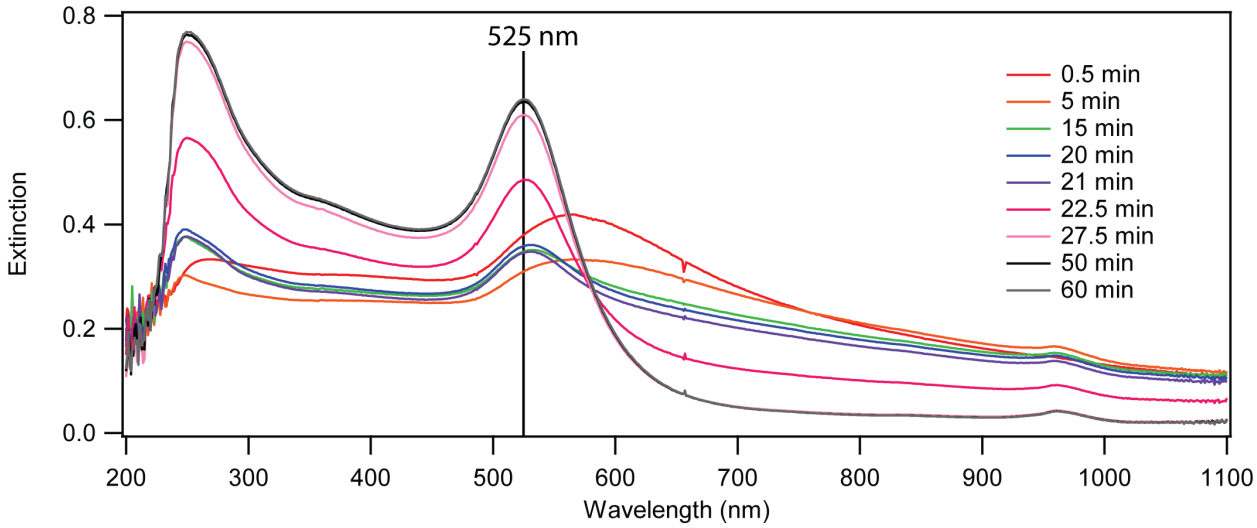

Figure S5: Representative spectra of key time points of a kinetic study of target induced disassembly of DNA-AuNP aggregates with no toeholds. At time zero, the sample was placed in the spectrometer at a temperature of T_m-2 (35.6 °C). The target was added at 20 minutes. The sharp rise in extinction at 525 nm between 21 minutes (just after target addition) and 27.5 minutes illustrates the liberation of DNA-AuNP colloids by strand displacement. The spectral changes in the first 20 minutes represent the thermal equilibration of the aggregates upon heating them from room temperature, where they were formed, to T_m-2.

Visualization Experiments:

For the direct visualization experiments, 1 mL of preformed aggregates (with oA_5 linker) was taken and tabletop centrifuged (Labnet International Inc., Catalogue # C1301) for ~5 seconds. The supernatant (900 µL) was pipetted out and the remaining 100 µL of aggregate and buffer was lightly vortexed and aliquoted in 10 µL proportions into separate eppendorfs. Afterwards, 10 µL of target (oT_5) of varying amounts as shown in Figure 5 was added to the sample, shaken lightly and monitored for a period of 10 minutes before photographs were taken.

References:

(1) OligoAnalyzer 3.1. https://www.idtdna.com/calc/analyzer (accessed November 30 2015).