Electronic Supplementary Information for:

Architecture based on the integration of intermolecular G-quadruplex structure with sticky-end pairing and colorimetric detection of DNA hybridization

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This document contains the following supplementary figures and information:

- Scheme S1. Signal transduction mechanism of preliminary colorimetric DNA assay.
- Figure S1. The dependence of capturing DNA-functionalized GNPs aggregation on the sequence of HDNA.
- Figure S2. The comparison of target DNA hybridization-induced GNPs aggregation degree of colorimetric assay systems containing different recognition probes.
- Figure S3. The influence of G-HDNA7 concentration on the UV-vis extinction spectrum of colorimetric system.

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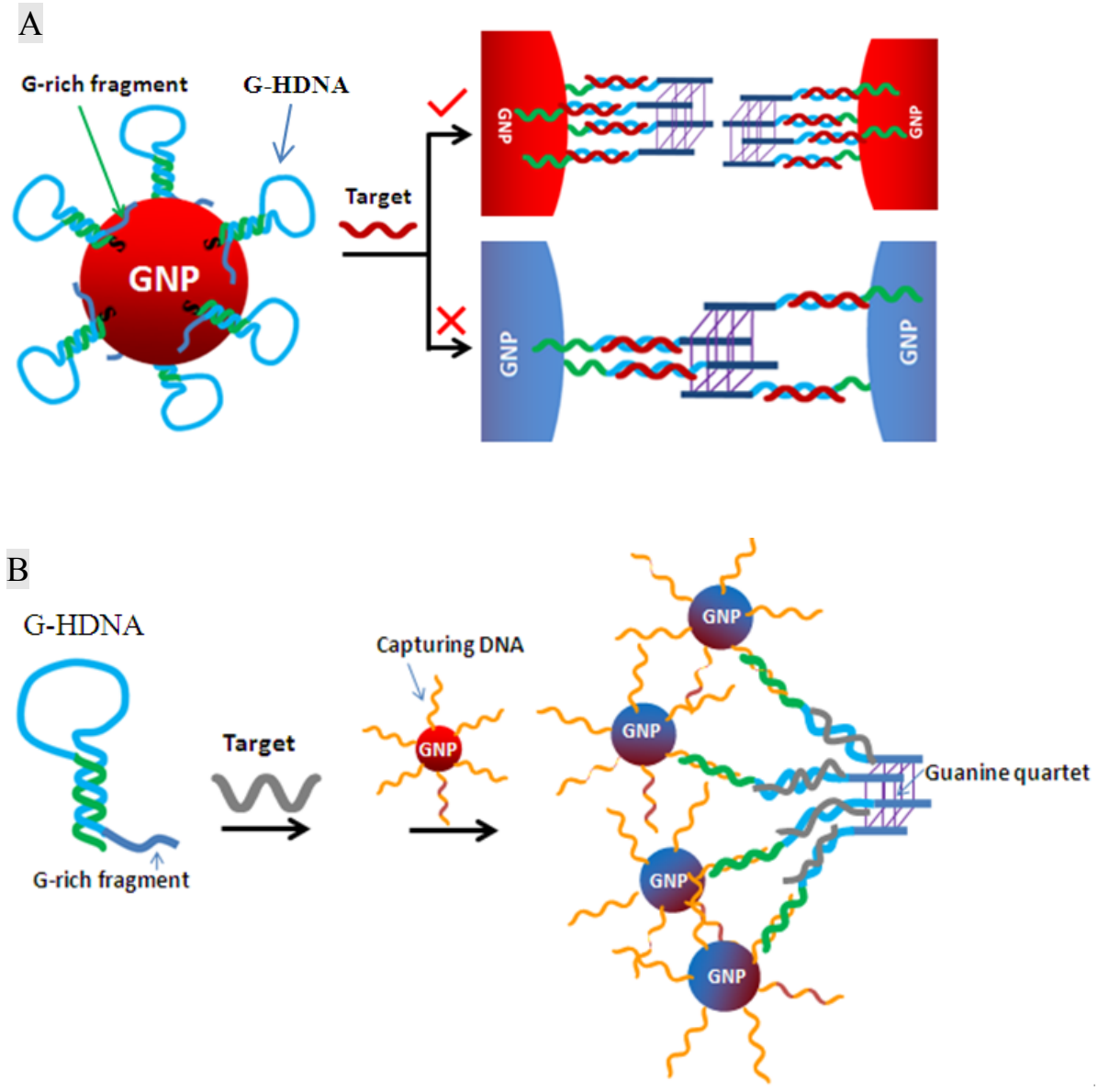

Scheme S1. Signal transduction mechanism of preliminary colorimetric DNA assay. **A)** Sensing design of GNP-confined signaling G-HDNA that could not only identify target DNA but also was expected to report the hybridization event. According to the previous reports on the anti-parallel G-quadruplex-induced GNP assembly [1,2], the hybridization of target DNA to the G-HDNA was thought to lead to the formation of the intermolecular

anti-parallel G-quadruplex structure after the opening of the hybridized stem that exposes the G-rich terminal region, causing the GNP aggregation. In contrast, the G-rich fragment of HDNA is deemed to be pulled by the stem of hairpin onto the GNP surface in the absence of target DNA, and the subsequent reactions were prevented. However, in fact, this target-triggered process cannot occur (data not shown). Even if the different G-HDNA sequences were tried, no obvious change in the solution color upon DNA target hybridization was observed. Our very recent studies [3,4] demonstrate that nucleic acids with a long G-rich terminal fragment per one strand can form an intermolecular parallel, rather than anti-parallel, G-quadruplex structure. Thus, it is reasonable that DNA-functionalized GNPs do not aggregate because of the lack of cross-linking interaction between them and the increase in electrostatic repulsion resulting from the hybridized target DNA. The red tick and cross in this illustrative scheme indicate the actual and imaginary reaction processes, respectively. **B)** Anticipated operation mode of adapted colorimetric system where the recognition reaction and signaling process are separated. The integrated G-HDNA is broken into two probes: target-binding G-rich hairpin DNA (G-HDNA) and capturing DNA attached onto gold nanoparticles (GNPs). The hybridization between G-HDNAs and capturing DNAs is inhibited by the formation of stem in the absence of target DNAs. However, the hybridization of target DNAs to G-HDNAs is expected to not only release the fragment complementary to capturing DNA but also promote the assembly of G-quadruplex structure, leading to the aggregation of GNPs that induces the solution color change from red to blue.

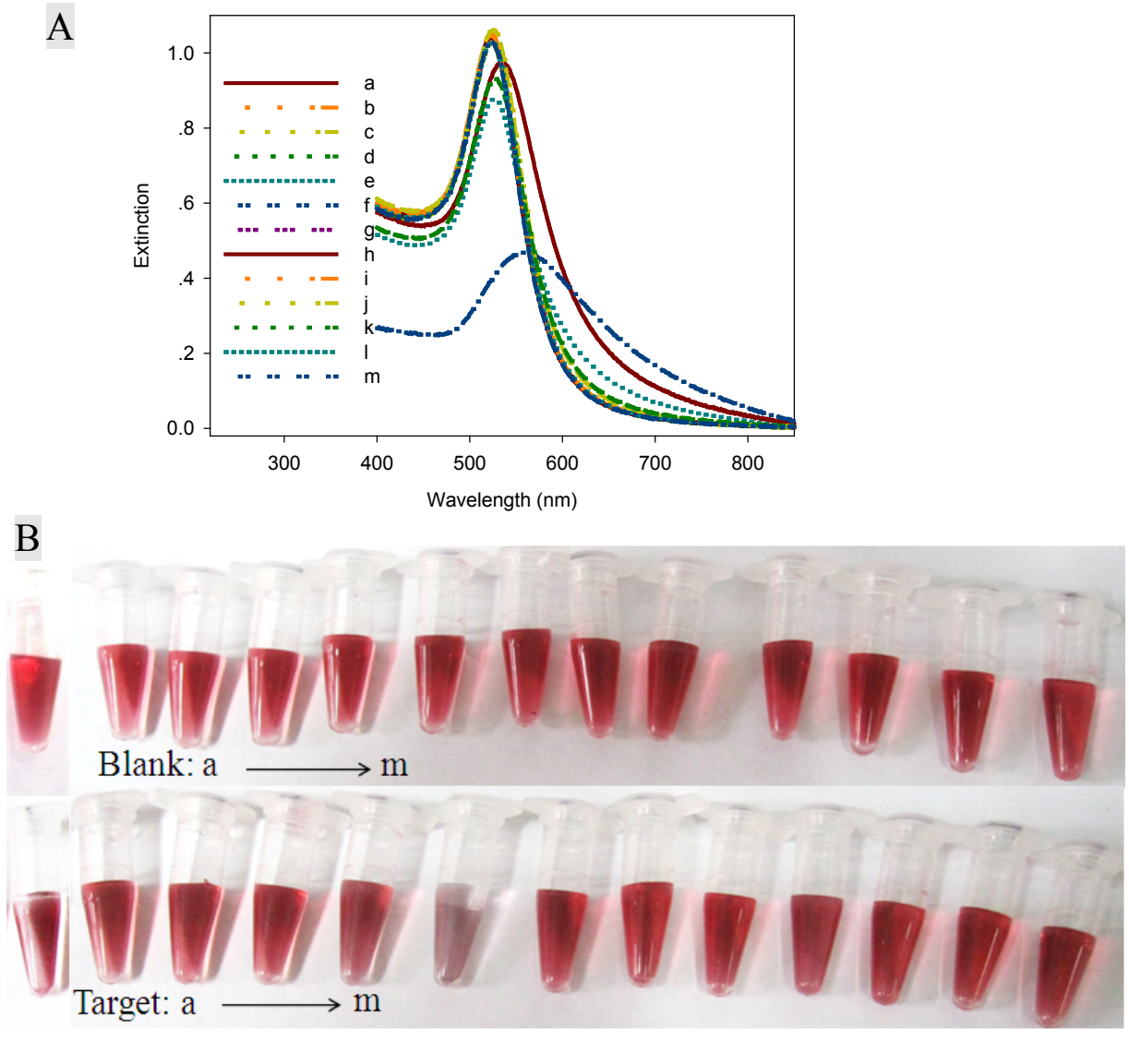

Figure S1. The dependence of capturing DNA-functionalized GNPs aggregation on the sequence of HDNA. (A) Extinction spectra collected for the mixture of capturing DNA-functionalized GNPs and different HDNAs in the presence of target DNA; B) Photographs of G-quadruplex-based colorimetric system in the absence (Blank) and presence (Target) of target DNA. Tubes a to m indicate the colorimetric systems containing G-HDNA1 to G-HDNA4, G-HDNA6 to G-HDNA10, C-HDNA1, C-HDNA2, G-HDNA5 and G-HDNA11, respectively. Target DNA at the concentration of 1 μM is used to evaluate the assay system. The data demonstrate that, only for the G-HDNA7-based assay system, the existence of target DNA can be detected by colorimetric measurements.

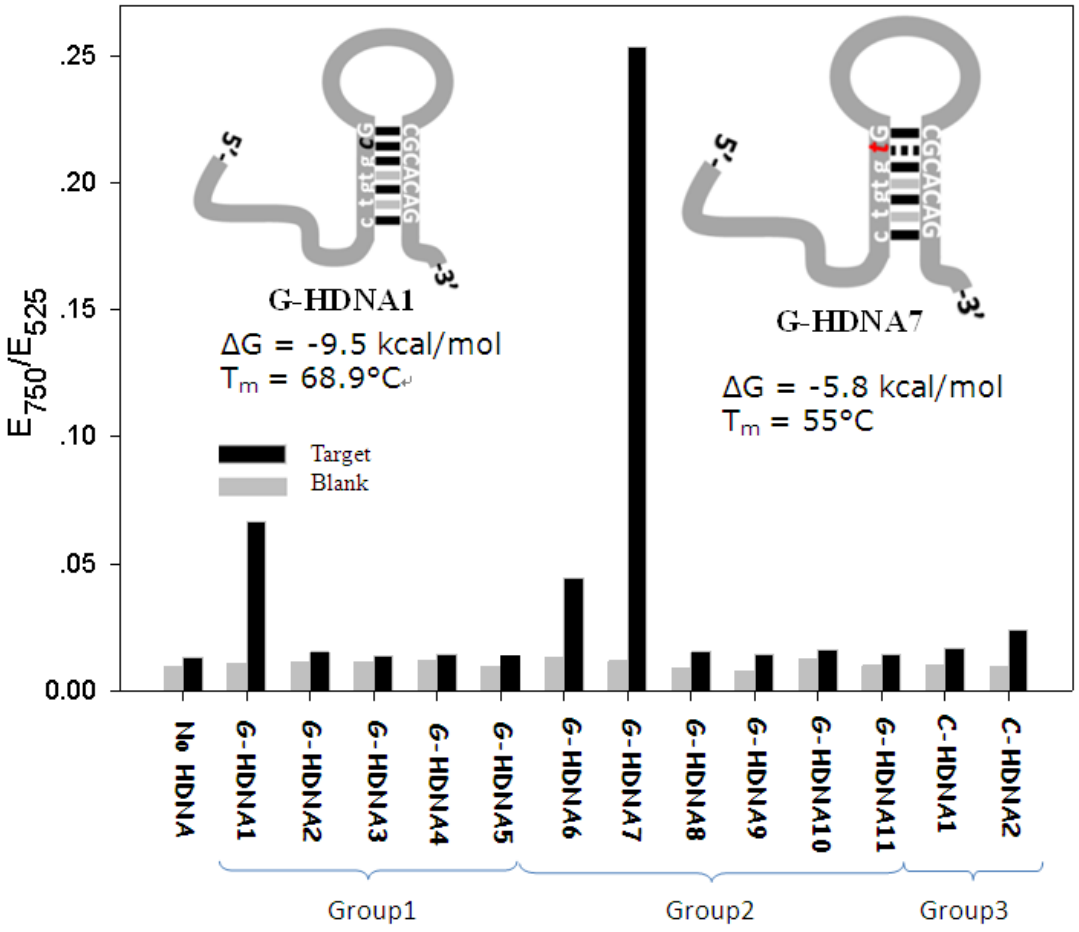

Figure S2. The comparison of target DNA hybridization-induced GNPs aggregation degree (evaluated by the ratio of UV-vis extinction peak at 750 nm to that at 525 nm) of colorimetric systems where G-HDNA7 or each of other HDNAs serves as target recognition probe. Target DNA at the concentration of 500 nM is involved in this section. **The inset:** the secondary structure, accompanied by the free energy change and melting temperature, of G-HDNA1 or G-HDNA7 that is predicted by the program "mfold" (http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/dna-form1.cgi). Compared with the G-HDNA1, the G-HDNA7 stem contains a non-Watson-Crick base pairing between T and G.

Dependence of Signal Transduction on G-HDNA Sequences

To explore the molecular mechanism of G-quadruplex-based colorimetric system for DNA detection and to prove the rationality of the choice of G-HDNA7 as recognition element, three groups of hairpin DNAs (including group1, group2 and group3) were designed and the detailed information of HDNA sequences is indicated in Table 1. The target-related UV-vis spectra, as well as the color changes observed by the naked eye in the absence and presence of target DNA, were collected in Figure S1. The corresponding analytical results are shown in Figure S2. The G-HDNA7-contained colorimetric system exhibits the strongest aggregation compared with all other sensing systems where different HDNAs were involved. For example, when removing two bases of the fragment with the gray background (complementary to the middle gray fragment and to the capturing DNA) away from the 3' end of G-HDNA (i.e., G-HDNA6), the signal substantially decreases, indicating that CP (capturing DNA)-modified GNPs do not aggregate. Namely, the G-HDNA cannot form the expected G-quadrulex structure and/or cannot efficiently hybridize to the GNP-confined capturing DNA even in the presence of target DNA. Similarly, if the G-HDNA is extended by several bases at the 3' end to have longer fragment complementary to capturing DNA (i.e., G-HDNA8 to G-HDNA11), target DNA hybridization does not induce the more intensive GNP aggregation but a relatively negligible change in UV-vis extinction spectra of colorimetric system compared with the blank. When the noncanonical base pair, G-T, in the stem of G-HDNA7 is change to G-C, the signal intensity is dramatically compromised regardless of the length of complementary fragment to capturing DNA (shown in G-HDNA1 to G-HDNA5 of Figure S2). Seemingly, this is determined by the intrinsic characteristics of G-HDNA probes. According to the

previous report [5], a classical HDNA contains the recognition sequence (usually 15-30 bases) complementary to a target DNA, and the self-complementary stem has 5-7 base pairs. Comparatively, the stem (7 base pairs consisting of five G-C pairs and A-T pairs) of G-HDNA1 is too stable to signal efficiently target DNA with 18 complementary bases, which can be indicated by the considerable difference in the free energy change between G-HDNA1 and G-HDNA7 (shown in Figure S2 Inset). If HDNA is designed to have no the stem-loop structure (C-HDNA1) or no the G-rich fragment (C-HDNA2), target DNA hybridization cannot make colorimetric system change significantly its UV-vis extinction spectrum. The measured data not only imply that the anticipated sensing scheme described in Scheme S1B (where the longer base fragment complementary to capturing DNA at the 3' end of G-HDNA cannot make the GNP solution keep dispersion status) is not the explanation for the observations, but also demonstrate that the stem, G-rich fragment and the region complementary to the capturing DNA play a very important part in the signal transduction of colorimetric system. This indicates the new mechanism responsible for the GNP aggregation.

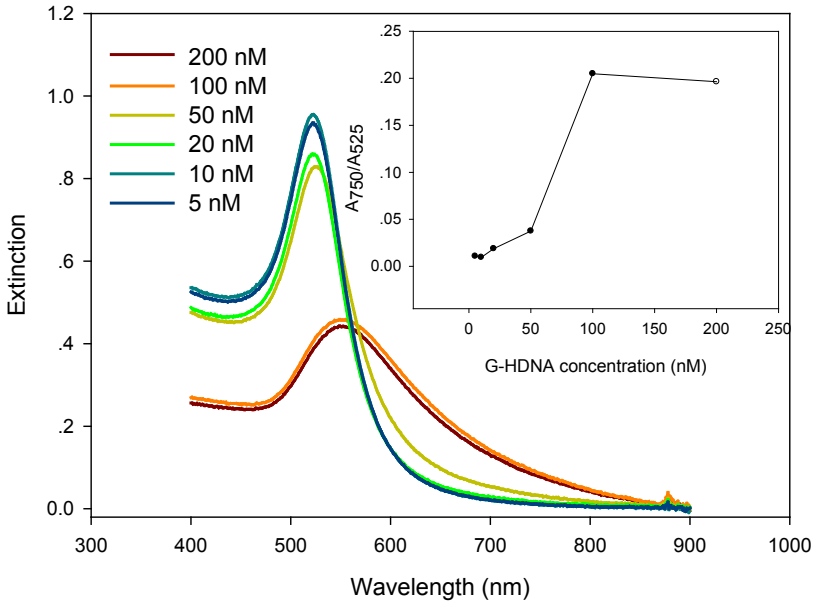

Figure S3. The influence of G-HDNA7 concentration on the UV-vis extinction spectrum of colorimetric system. The experiments were carried out as shown in the "Experimental Section". The target DNA concentration is 500 nM. **Inset:** the relationship between the extinction peak ratio of UV-vis spectrum and G-HDNA concentration.

Optimization of G-HDNA Concentration

For the colorimetric system, that target DNA hybridization opens the hairpin structure of G-HDNA is designed to promote the formation of G-quadruplex, and on the basis of sticky-end pairing effect, the resulting quadruplexes are capable of self-assembling into two or three dimensional architecture that causes the GNP aggregation via DNA hybridization. Naturally, the advanced DNA assembly starts from the premise that the amount of G-HDNA is sufficient. Thus, the dependence of target hybridization-triggered GNPs

aggregation on the concentration of G-HDNA is explored. As shown in Figure S3, no obvious characteristic of GNP aggregation is detected when G-HDNA7 concentration is lower than 50 nM. The reason is that the advanced structure of G-HDNA7/target duplex cannot be formatted when the G-HDNA7 concentration is low. In contrast, if the concentration of G-HDNA7 increases to 100 nM, the substantial change in UV-vis extinction spectra is observed, indicating the GNP aggregation. Figure S3 Inset depicts the relationship between the extinction peak recorded from UV-vis spectrum of colorimetric system in the presence of target DNA and G-HDNA concentration. Apparently, as long as the G-HDNA7 concentration is more than 100 nM, the hybridization of target DNA to G-HDNA can efficiently trigger the GNP aggregation.

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