Electronic Supplementary Material (ESI) for Analyst.

Supplementary materials for:

Collapse of chain anadiplosis-structured DNA nanowires for highly sensitive colorimetric assay of nucleic acids

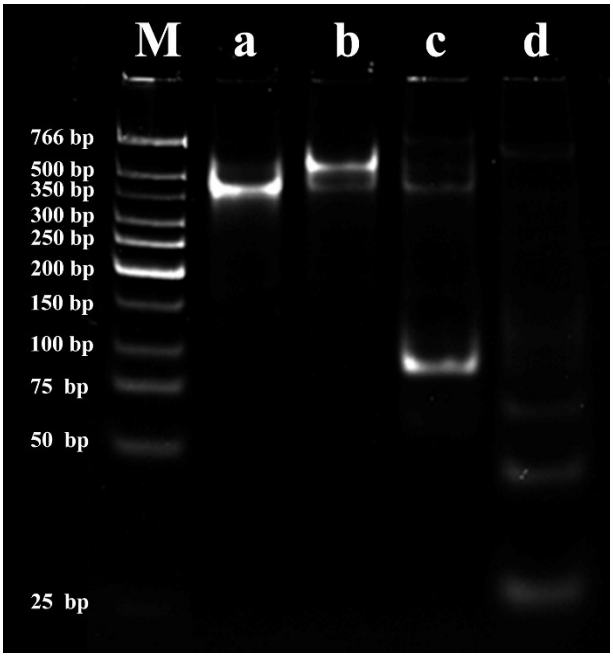

Figure S1. Results of 12% native-PAGE imaging analysis. (M) DNA marker; (a) AS1+AS2; (b) AS1+AS2+TP; (c) AS1+AS2+TP+Poymerase; (d) AS1+AS2+TP+Poymerase+Nickase. Experimental conditions: [AS1]= [AS2]= 250 nM; [TP]= 125 nM;

Gel electrophoresis characterization

To prove that the unique assembled nanowire can work as predicted, a set of experimental samples were characterized by polyacrylamide gel electrophoresis (PAGE). As evident in Figure S1, the assembly products of AS1 and AS2 appeared in lane a. Benefitting from the large number of

hybridized DNA nucleotide pairs-mediated chain anadiplosis assembly, the formatted nanowire was uniform and only one single bright band with slower migration rate was shown. The introduction of TP made the nanowire more retard (lane b), indicating that the TP hybridize with the nanowire. However, as seen in lane c, after addition of polymerase, the band representing the nanowire almost disappeared and one band with low molecular weight appeared. This phenomenon is attributed to the extension of TP over AS1, making the AS1 peel off from the nanowire. More importantly, the coexistence of polymerase and nickase has a marked effect on the structured nanowire. Not only does the band assigned to the nanowire almost completely disappeared, but also appeared several bands with fast mobility. All the results presented herein are in good agreement with the predicted principle, confirming the mechanism is reasonable.

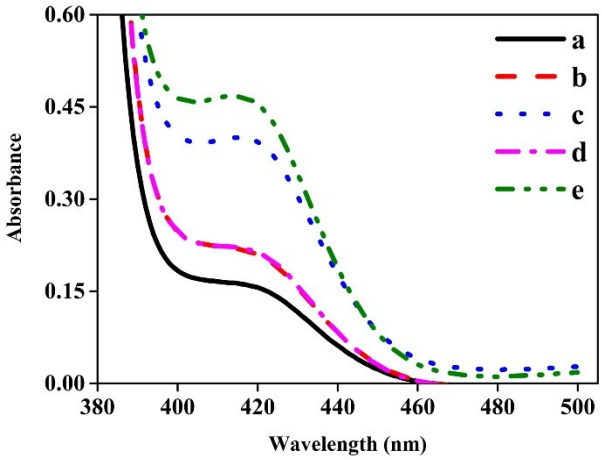

Figure S2. Typical UV-Vis spectra obtained from the oxidation of ABTS by H_2O_2 in the presence of hemin (a), GCF2 and hemin (b), GCF2, AS1, hemin, polymerase and nickase (c), GCF1 and hemin (d), GCF1, AS2, hemin, polymerase and nickase (e). Experimental conditions: [GCF1]= [GCF2]= 250 nM, [AS1]= [AS2]= 500 nM, [H_2O_2]= 50 μM, [Hemin]= 1.25 μM, Oxidation time= 30 min.

Catalytic activity and amplification effect of GCF1 and GCF2

To check the catalytic activities of GCF1 and GCF2 and to affirm the amplification effects of GCF1/AS2 and GCF2/AS1 complexes, we examined the corresponding absorption spectra of different samples under identical conditions. As described in Figure S2, the absorption intensity of GCF1 (curve d) and GCF2 (curve b) mediated signal output is evidently stronger than the inherent signal ascribed to the oxidization of ABTS by H_2O_2 without any DNAzymes (curve a), implying the catalytic activity of GCF1 and GCF2. Interestingly, compared with curve b, the mixture of GCF2/AS2 coexisting with the polymerase and nickase exhibits a markedly enhanced signal (curve c), and similar outcome occurs in comparison of curve e with curve d, suggesting the polymerization/nicking/displacement based accumulation of G-quadruplexes for signal amplification achieved in the duplexes of GCF1/AS2 and GCF2/AS1.

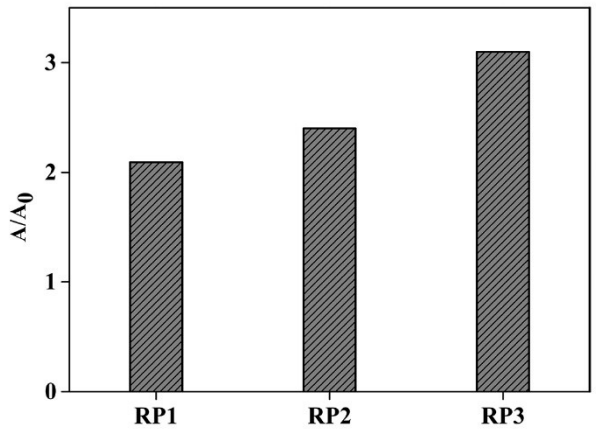

Figure S3. Histogram of the ratios of A/A_0 related to three different RPs upon detection of 75 nM kras gene. A and A_0 are the absorption intensity in the presence and absence of target DNA, respectively. Experimental conditions: [RP1]= [RP2]= [RP3]= 250 nM, [H_2O_2]= 50 μM, [Hemin]= 1.875 μM, [ABTS]= 0.9 mM. Oxidation time= 20 min.

Selection of RPs

Prior to evaluate the assay performance of the proposed chain anadiplosis-structured DNA nanowire for kras gene sensing, three different RPs were projected to carry out signal transduction. The ratio of A/A_0 in the absorption intensity after and before adding kras gene was used to estimate the signal intensity. As seen in Figure S3, the signal amplification achieved by RP3 is better than RP1 and RP2, which was attributed to the nucleotide changes in RPs design. As detailed in Table 1, the stem of RP3 consist of 13 pairs of self-complementary bases, which is stable responsible for the low background absorption. Additionally, compared with RP1 and RP2, RP3 have 13 bases complementary to AS1, making RP3 easier to implement the signal amplification. Accordingly, the RP3 is preferred to gain the best sensing performance.

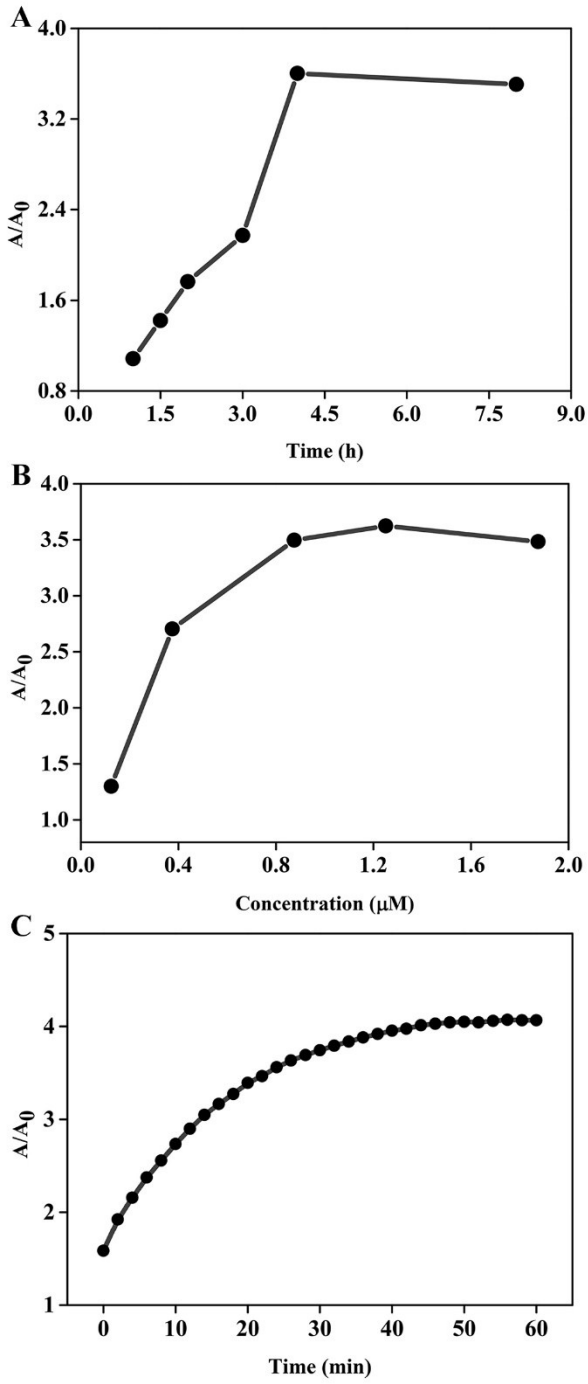

Figure S4. Optimization of experimental conditions for target DNA detection. (A) Relationship between the ratio of A/A_0 and different enzymatic reaction time. (B) Effect of the concentration of hemin upon the signal intensity. (C) Dynamic variations of A/A_0 collected from different oxidation time. The target concentration is 75 nM.

Optimization of the experimental conditions for gene detection

After the chain anadiplosis-structured DNA nanowire was confirmed to be capable of being extended for gene sensing, several experimental parameters such as the effect of enzymatic reaction time, the concentration of hemin, and the oxidation time of ABTS were investigated. The value of A/A_0 was adopted, where A and A_0 are the absorption intensity in the presence and absence of target analyte, respectively. Figure S4A depicted the influence of enzymatic reaction time on the signal output. It was found that the value of A/A_0 increases in the time ranging from 0 to 4 h and then reached to a constant after 4 h. We thus selected 4 h as the optimal enzymatic reaction time. Figure S4B shown that the value of A/A_0 has an ascent in the low hemin concentration range, and the maximum value appears when the concentration of hemin increases to 1.25 μM. This observed phenomenon is reasonable taking into account that at the low concentration of hemin, the formation of DNAzymes is limited, therapy causing insufficient signal response. However, high concentration of hemin would inevitably lead to a relative high background, yet compromising the assay ability. Therefore, we choose 1.25 μM as the favored one for DNAzymes formation. Additionally, as shown in Figure S4C, the kinetic curve of A/A_0 versus the oxidation time of ABTS increased in a time dependent manner and hardly changed when the time reached to 40 min, reflecting that the reaction was thoroughly executed. Thus, the incubation time of 40 min is suitable for colorimetric response.