Electronic Supplementary Information

A Centrifugation-assisted Visual Detection of SNP in Circulating Tumor DNA using Gold Nanoparticle Coupled with Isothermal Amplification

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Materials and Methods

Table S1. DNA oligonucleotides used in this study

Item	**DNA oligonucleotide sequence**
wtKRAS	ACCTCTATTGTTGGATCATATTCGTCCACAAAATGATTCTG AATTAGCTGTATCGTCAAGGCACTCTTGCCTACG**C**CACCAG CTCCAACTACCACAAGTTTATATTCAGTCATTTTCAGCAGG CCTTATAATAAAAATAATGAAAATGTGA
mtKRAS	ACCTCTATTGTTGGATCATATTCGTCCACAAAATGATTCTG AATTAGCTGTATCGTCAAGGCACTCTTGCCTACG***T***CACCAG CTCCAACTACCACAAGTTTATATTCAGTCATTTTCAGCAGG CCTTATAATAAAAATAATGAAAATGTGA
mLinker	5'-GAGCTGGTG***A***CGTAGGCA-3'
c-mLinker	5'- TGC CTA CG***T*** CAC CAG CTC -3'
OA	5'-CAC CAG CTC CAA CT-(A)10-S-S-3'
OB	5'-S-S-(A)10-ACT CTT GCC TAC GT-3'
c-OA	5'-S-S-(A)10-AGT TGG AGC TGG TG -3'
c-OB	5'-ACG TAG GCA AGA GT-(A)10-S-S-3'

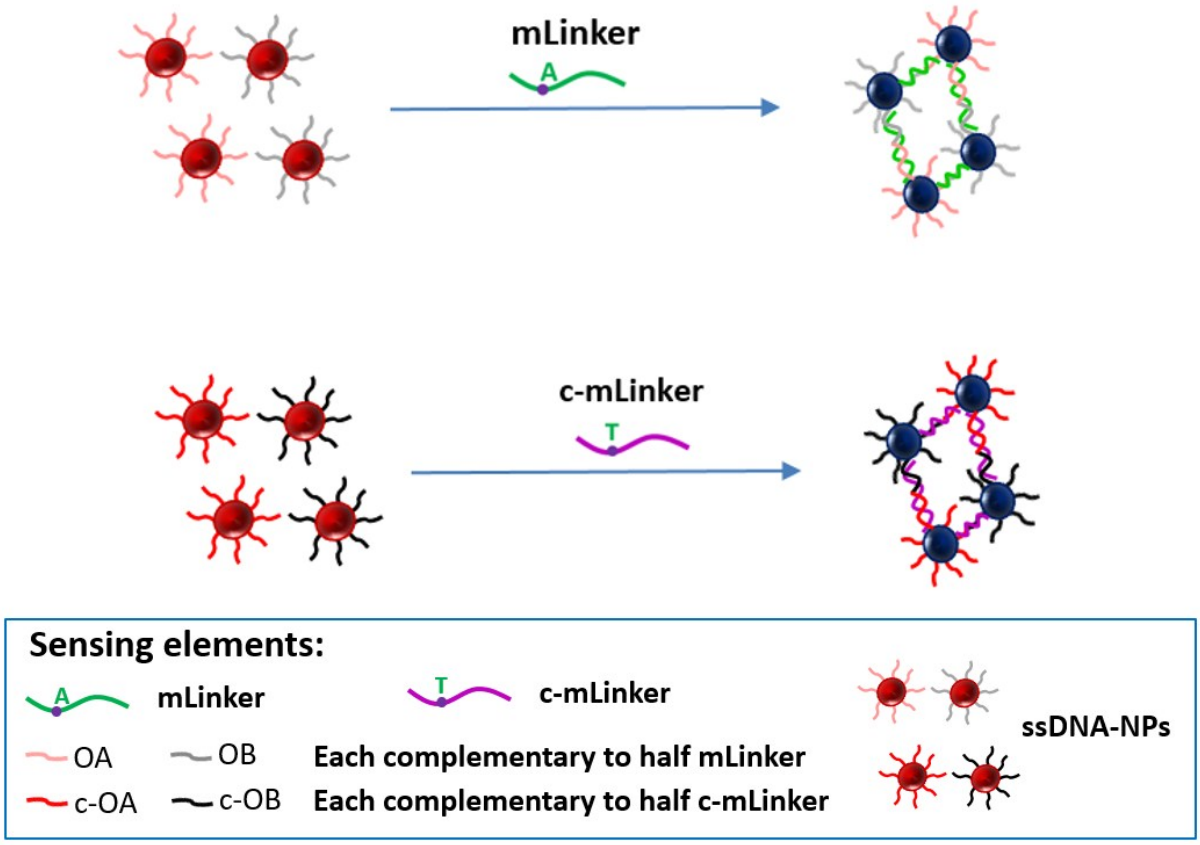

Fig. S1. Schematic illustration of sandwich assembly of ssDNA-NPs induced by the mLinker or c-mLinker in this study.

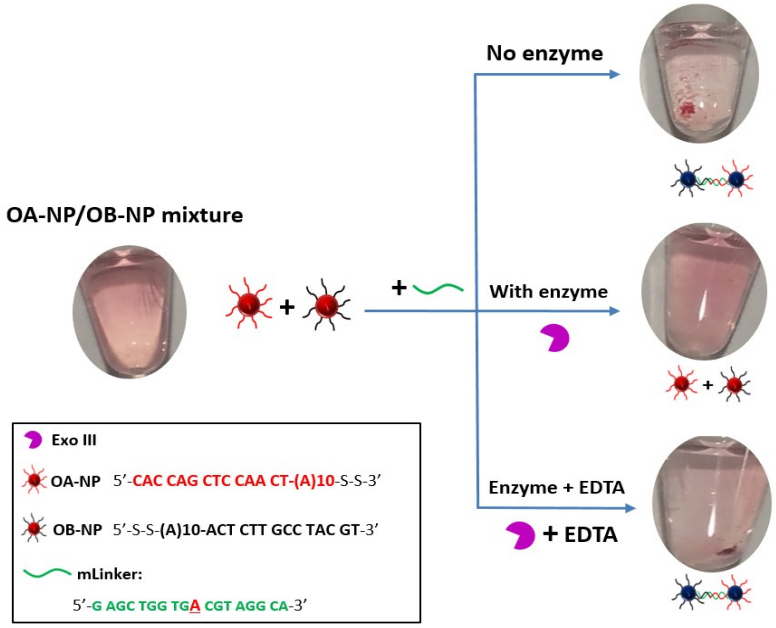

Fig. S2. Enzyme inactivation by EDTA treatment at room temperature. Note that the mLinker–induced sandwich ssDNA-NPs assembly (including OA-NP and OB-NP, each with ~2 nM in the final solutin) was done in PBS buffer (150 mM). The mixture was incubated for less than 30 min followed by a brief centrifugation step (6000 rpm, ~10 sec).

Note: We conducted three expriments to confirm the role of EDTA to inactivate ExoIII. In the first experiment, the ssDNA-NP conjugates (OA-NP and OB-NP) were mixed with the mLinker DNA, without addition of ExoIII. The ssDNA-NP mixture changed from the red dispersion state into precipitated aggregates as expected, because of the formation of interparticle bridging DNA hybrids among the OA, OB and the mLinker DNA. In the second experiment, when the mLinker DNA probe was added into the ssDNA-NP mixture, together with the enzyme, the solution remained in red dispersion. This should be due to the fact that ExoIII digests the linker DNA from 3' to 5' on the asformed DNA duplex, thus causing ssDNA-NP solution stays in dispersed red state. In the third experiment, EDTA was mixed with mLinker and ExoIII before adding into the ssDNA-NP mixture solution. After 3 min incubation at RT, the ssDNA-NP mixture showed precipitated aggregate state. In addition, pure addition of EDTA into the solution mixture (OA-NP, OB-NP, and mLinker) did not affect the ssDNA-NP sandwich assembly (data not shown). With these observations, we concluded that EDTA had effectively quenched the enzymatic activity of ExoIII at RT after 3 minutes.

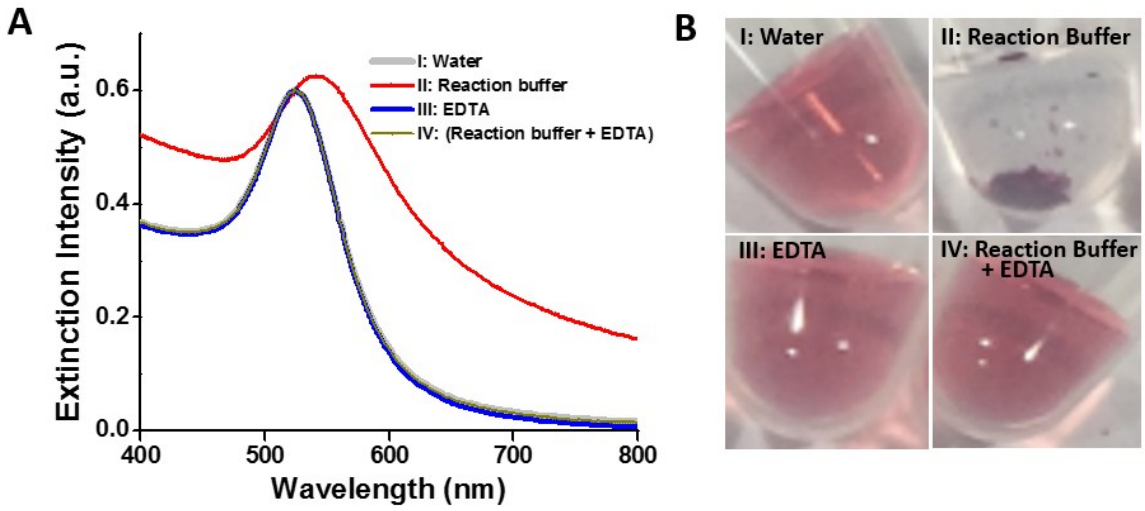

Fig. S3. **Effect of EDTA on colloidal stability.** (A) UV-Vis spectra of the incubation solution of ssDNA-NP conjugates (including OA-NP and OB-NP, each with ~4.8 nM in the final solutin) in different medium before spinning, where I (water), II (8 µl enzyme reaction buffer), III (2 µl 500 mM EDTA), and IV (both 8 µl enzyme reaction buffer and 2 µl 500 mM EDTA). (B) camera images of ssDNA-NP solution after a brief spinning (6000 rpm, ~10 sec).

Note: The reaction buffer, including Tris-HCl buffer 20 mM, 20 mM Ca^{2+} and 3 mM Mg^{2+}, provides a conducive environment and is essential for ExoIII structure-selective digestion of mLinker in the mLinker-mtKRAS complex.[1] However, this buffer medium (especially the strong ionic strength) can cause ssDNA-NP aggregation even in the absence of linker probe. Interestingly, applying EDTA in the enzyme inactivation step not only inactivates the enzyemm, but also maintains the above colloidal stability**.** As shown in **Fig. S3,** ssDNA-NP conjugates themselves become aggregated in the enzymatic reaction buffer, as shown by the red shift of the LSPR peak and the broaden band when compared to them in water. After the centrifugation, large amount of precipitation is observed (**Fig. S3B**, image II). However, in the presence of EDTA, the ssDNA-NP remain stable, even when reaction buffer is added (overlapping UV-Vis curves with that in water). The solution can even withstand spinning (**Fig. S3B**, images III and VI), same as that in water (**Fig. S3B**, image I). These observations confirmed that the enzymatic reaction buffer tends to introduce unwanted particle aggregation, but addition of EDTA can stablize the colloidal system in presence of enzyme reaction buffer.

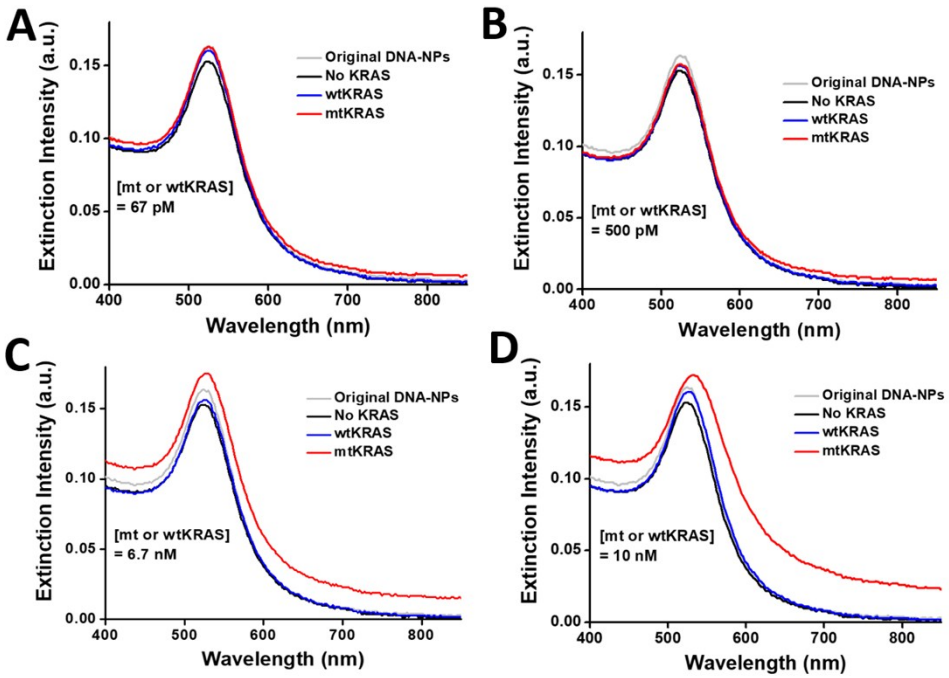

Fig. S4. UV-Vis spectra of ssDNA-NP assembly after 30 minute incubation in the enzyme reaction solution. wtKRAS or mtKRAS with different concentration including A) 67 pM, B) 500 pM, C) 6.7 nM, and D)10 nM. A sample with No KRAS was used as the control for comparison.

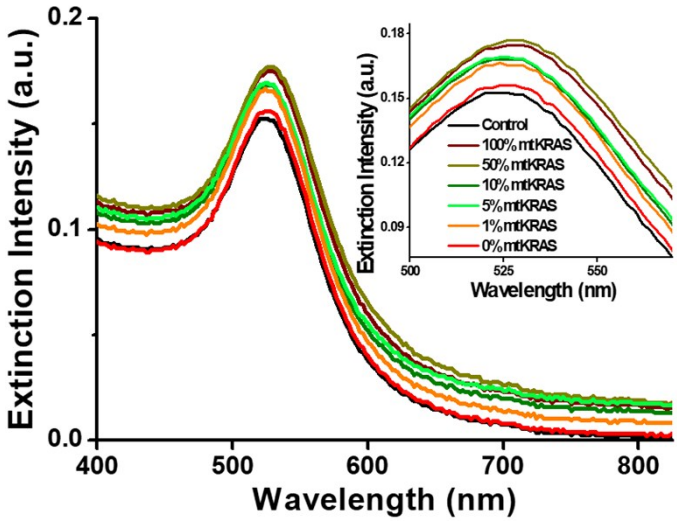

Fig. S5. UV-Vis detection of mtKRAS using c-mLinker system. The percentage of mtKRAS relative to the total ctDNA (mtKRAS + wtKRAS) is 0%, 1%, 5%, 10%, 50%, and 100%. A sample with No KRAS was used as Control for comparison.

Reference

1 S. Wu, P. Liang, H. Yu, X. Xu, Y. Liu, X. Lou, Y. Xiao, Amplified single base-pair mismatch detection via aggregation of exonuclease-sheared gold nanoparticles, Anal. Chem. 86 (2014) 3461-3467. https://doi.org/10.1021/ac4040373.