Supplementary information

A Novel Label-Free Capillary Electrophoresis LED-Induced Fluorescence Platform Based on Catalytic Hairpin Assembly for Sensitive Detection of Multiple Circulating Tumor DNA

Yanyan Sun[§], Si He[§], Yufei Peng[§], Min Liu[§], Danke Xu[§,*]

§: State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, No 163, Xianlin Avenue, Nanjing, 210023, PR China

*: corresponding author, Tel: +86-025-89685835, Email: xudanke@nju.edu.cn

Table S1 DNA oligonucleotide sequences involved in this work

Name	Sequences (5'-3')
H1-PI	ATCCTCTCTCTAAAATCACTGAGGTACGCTCTCAGTGATTTTA
H2-PI	ACTGAGAGCGTACCTCAGTGATTTTAGTACGCT
PIK3CA	CTCAGTGATTTTAGAGAGAGGAT
H1-TP	TGGGGGCAGCGCCTCACAACCTCCCATGTGTAGAGAGGTTGTGAGGCG
H2-TP	ACAACCTCTCTACACATGGGAGGTTGTGAGGCGCCATGTGTAGA
TP53	GAGGTTGTGAGGCGCTGCCCCCACCATG
MT1-PI	CTCAGTGATTTTAGTGAGAGGAT
MT1-TP	GAGGTTGTGAGGCGCTGCGCCCACCATG
MT3-PI	CTCTGTCATTTTAGTGAGAGGAT
MT3-TP	GAGGTTGAGAGGCGGTGGCCCCACCATG
Random	GTAGCTTATCAGACTCGACTTAGATGT

Table S2 ΔG of all hairpin probes and hybrid complexes in the CHA system

Targets	Name	ΔG (kcal/mol)
	H1-PI	-8.7
	H2-PI	-4.53
PIK3CA	PIK3CA	-2.91
	H1-PI/PIK3CA	-22.94
	H1-PI/H2-PI	-30.76
	H1-TP	-14.14
	H2-TP	-7.77
TP53	TP53	-1.92
	H1-TP/TP53	-32.51
	H1-TP/H2-TP	-43.12

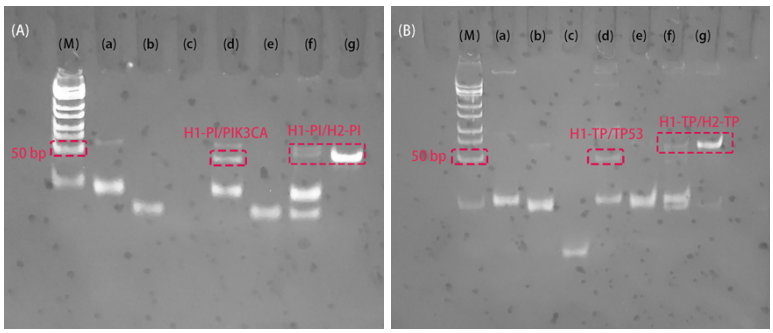

Figure S1 Gel electrophoresis analysis of the CHA system for PIK3CA and TP53. (A) Lane a~g: H1-PI, H2-PI, PIK3CA, H1-PI+PIK3CA, H2-PI+PIK3CA, H1-PI+H2-PI, H1-PI+H2-PI+PIK3CA. Lane M: Marker (25 bp~500 bp). (A) Lane a~g: H1-TP, H2- TP, TP53, H1- TP +TP53, H2-TP+TP53, H1- TP+ H2- TP, H1- TP+ H2- TP+TP53. Lane M: Marker (25 bp~500 bp).

Table S3 Comparison with MCE based DNA detection methods

Method	Analyte	Detection	LOD	Ref.

		range		
Isothermal strand-displacement polymerase reaction	mecA gene	1-100 nM	12.3 pM	[1]
Defective T junction-mediated strand displacement amplification	16S rDNA	10 pM-10 nM	10 pM	[2]
Crispr-Cas12a enzyme-derived nucleic acid detection	16S rDNA	100 pM-500 nM	45 pM	[3]
Catalytic hairpin assembly reaction	PIK3CA	50 pM-1 nM	20.35 pM	This work
	TP53	50 pM-1 nM	19.61 pM	

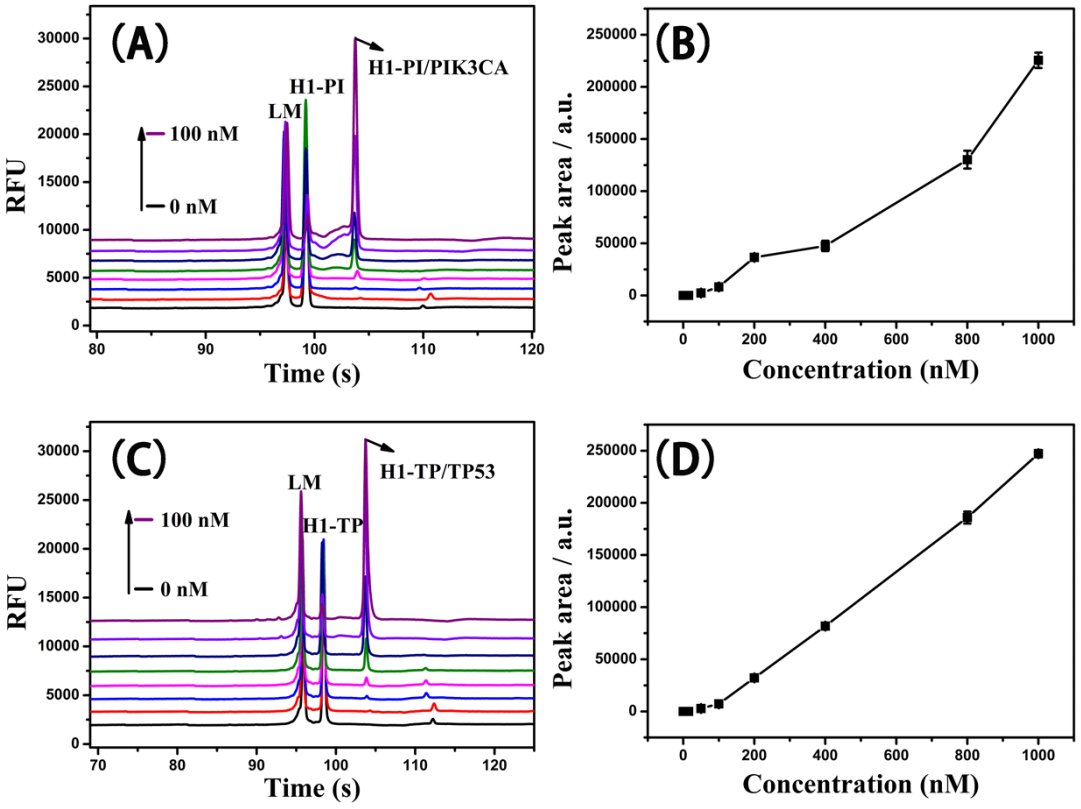

Figure S2 (A) and (C) the non-CHA amplification NGCE assay results. (A) PIK3CA, (C) TP53. (B) and (D) corresponding calibration curve to different concentration of targets PIK3CA and TP53, respectively (0,15/20,50,100,200,400,800,1000 nM)。(error bar：n=3)

Table S4 ΔG of H1/ctDNA(base mismatch) complexes

Targets	Name	ΔG (kcal/mol)

PIK3CA	H1-PI/MT1-PI	-20.25
	H1-PI/MT3-PI	-17.7
TP53	H1-TP/MT1-TP	-30.27
	H1-TP/MT1-TP	-26.26

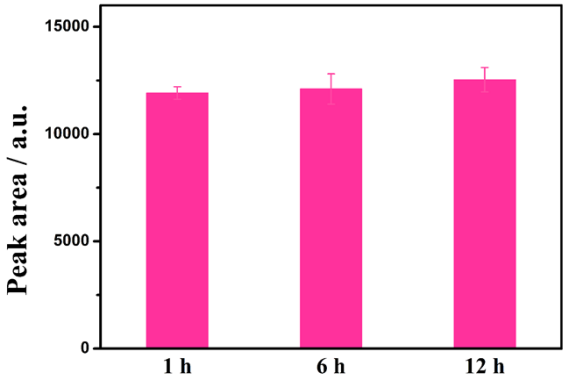

Figure S3 NGCE-LEDIF analysis of CHA complex of PIK3CA system after incubated for different time.

References

[1] Y. Lu, F. Luo, Z. Li, G. Dai, Z. Chu, J. Zhang, F. Zhang, Q. Wang and P. He, *Talanta*, 2020, **222**, 121686.

[2] F. Luo, Y. Lu, X. Geng, Z. Li, G. Dai, Z. Chu, J. Zhang, F. Zhang, P. He and Q. Wang, *Analytical Chemistry*, 2021, **93**, 3551-3558.

[3] F. Luo, X. Geng, Z. Li, G. Dai, Z. Chu, P. He, F. Zhang and Q. Wang, *RSC Advances*, 2022, **12**, 22219-22225.