Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

A Smart Fluorescence Biosensor for Highly Sensitive Detection of BRCA1 Based on 3D DNA Walker and ESDRs Cascade Amplification

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Table S1. Oligonucleotides used in the present work

Name	Oligonucleotide sequences (5′- 3′)
BRCA1	GAGCATACATAGGGTTTCTCTTGGTTT
PP	TGAGGCCAAGAGAAACCCTATGTATGCTC
DWTs	Biotin-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
	TTTTTTATAGGGTTTCTCTTGGCCTCA* GC
SDs	Biotin-TTTTTT GC*T GAGGTAGAGATTTTCCACAT
SDs-F	Biotin-TTTTTT GC*T GAGGTAGAGATTTTCCACAT-FAM
P	FAM-CCTACGTCTCCAACTAACTTACGG
S	CCCTTGAGGTAGAGATTTT
A	CCTACGTCTCCAACTAACTTACGGCCCTTGAG
	GTAGAGATTTT
L	ATGTGGAAAATCTCTACCTCAAGGGCCGTAAGTTAGTTGGA
	GACGTAGG-Dabcyl
K-ras	GTTGGAGCTGGTGGCGTAGGCAAGAGTG
P53	CAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTG
PIK3CA	GGAGTATTTCATGAAACAAATGAATGATGCACATCATGGTG
	GCTGGACAACAAAAATGGATTGGATCT

Blue region represents the nicking recognized sequence, "*" represents the cutting site of Nb.BbvCI.

Table S2. Comparison of different BRCA1 detection methods

Platform [a]	Strategy [b]	Dynamic range	LOD	Reference
Colorimetric	3D DNA nanostructured reporter probe	10 fM - 10 pM	10 fM	1
Electrochemical	Electrospinned ribbon conductive nanofibers	5 pM - 14 nM	2.4 pM	2
Fluorescence	Water-soluble conjugated polythiophenes	10 pM - 1 nM	2.2 pM	3
EIS	λ exonuclease assisted target recycling amplification	0.1 nM - 10 nM	0.05 nM	4
Fluorescence	Walker and ESDRs	100 fM - 10 nM	41.4 fM	**This work**

[a] EIS, electrochemical impedance spectroscopy

[b] ESDRs, entropy-driven strand displacement reactions

Table S3. Recoveries of the constructed fluorescence biosensor via spiking targets in salmon sperm DNA

Sample no.	Spiking value (pM)	Assayed value (pM)	Recovery (%)	CV[a] (%)
1	1.0	0.973	97.3	5.0
2	10	10.2	102.0	7.1
3	100	100.7	100.7	7.5

[a] CV, coefficient of variation.

Fig. S1.

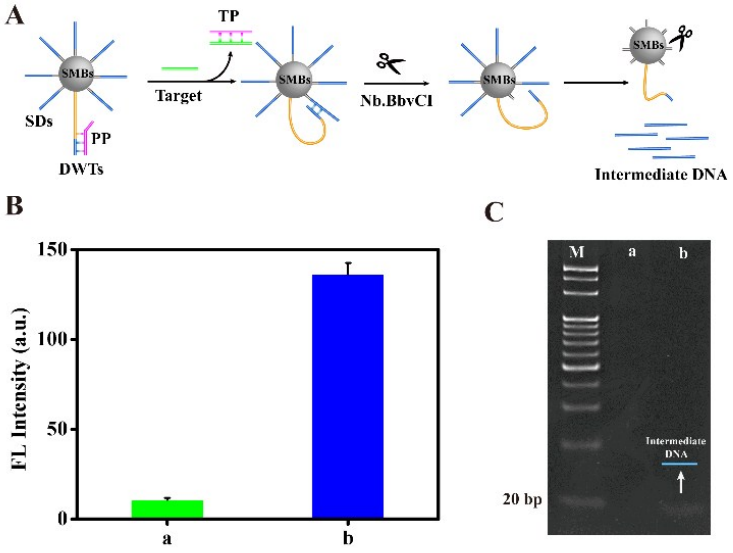
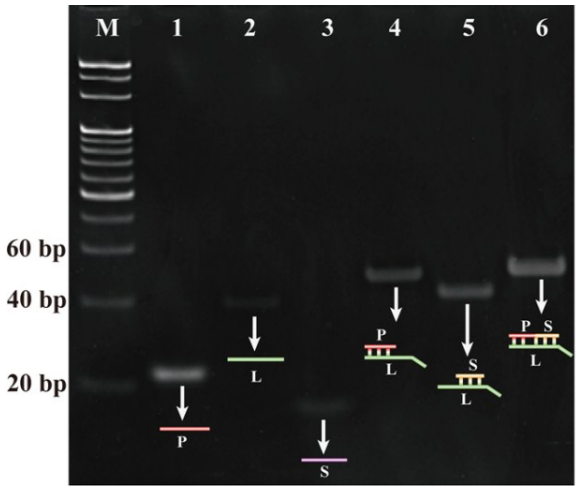


Fig. S1 (A) Schematic illustrating the mechanism of the DNA walkers. (B) Fluorescence analysis of products of the 3D DNA walkers: (a) in the absence of target DNA, (b) in the presence of 10 nM target DNA. (C) Native polyacrylamide gel electrophoresis analysis of the product of the DNA walkers. The lanes a: in the absence of target DNA, lane b: in the presence of 10 nM target DNA, lane M: 20 bp DNA size marker.

At first, the movement of DNA walkers were analyzed through a fluorescence method (Fig. S1B). Only weak signal was detected if the target DNA was absent (column a). On the contrary, if the target DNA and Nb.BbvCI is present, a mass of intermediate DNA modified with fluorescent group FAM, could be produced through the cleavage of the Nb.BbvCI and hence cause an intense fluorescence response (column b). In addition, the products of 3D DNA walker were also analyzed by native polyacrylamide gel electrophoresis. As shown in Fig. S1 (C), no band appeared without the addition of target DNA (lane a). However, one obvious band, the free

intermediate DNA, was observed once the target DNA was added (lane b). Theoretically speaking, there should be two bands in lane B, but the strand TP was disappeared which may blame to insufficient concentration. These results indicate that (1) the DWTs can be successfully activated in the presence of target DNA and (2) the DNA walking machine is well established.

Fig. S2.



Lane M: 20 bp DNA ladder marker; lane 1: 1 μM of strand P; lane 2: 1 μM of strand L; lane 3: 1 μM of strand S; lane 4: 1 μM of P-L complex; lane 5: 1 μM of S-L complex; lane 6: 1 μM of P-L-S complex.

To confirm the successful construction of the P-L-S three-stranded complex probes, 8% of native polyacrylamide gel electrophoresis was performed at 100 V constant voltage for 40 min. According to Fig. S2, Lane M was DNA ladder marker. In lane 1, there was strand P, while strand L existed in lane 2. However, the location of P-L complex (lane 4) is notably increased in gel compared with only strand P or strand L. Furthermore, stand S is located in lane 3. Compared with only strand L or strand S, the movement of S-L complex (lane 5) is slower. In contrast other lanes, the

site of P-L-S complex (lane 6) is higher because the molecular weight of P-L-S is greater.

References

1. X. Yang, Y.L. Wen, L.L. Wang, C.Q. Zhou, Q. Li, L. Xu, L.Y. Li, J.Y. Shi, R. Lal, S.Z. Ren, J. Li, N.Q. Jia and G. Liu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 38281-38287.

2. H. Ehzari, M. Safari, M. Shahlaei, *Microchem. J.*, 2018, **143**, 118-126.

3. Y. Ma, Y. Xia, L.Q. Yan, F. Wang, Z.H. Miao, M.F. Cui, H.T. Yao and Z.J. Qi, *Anal. Methods,* 2015, **7**, 5814-5819.

4. H.F. Xu, L.L. Wang, H.Z. Ye, L.S. Yu, X. Zhu, Z.Y. Lin, G.W. Wu, X.H. Li, X.X. Liu and G.N. Chen, *Chem. Commun.*, 2012, **48**, 6390-6392.