Supporting Information

Molybdenum disulfide@5-carboxyfluorescein-probe biosensor for unamplified specific fragment detection in long nucleic acids based on magnetic composite probe-actuated deblocking of secondary structure

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Table S1 Oligonucleotides employed in the present work

Oligonucleotides	Sequence (5'-3') [a]
PML probe	*TTCATAAGCTTGAGACAA*TTCACTGGCCACGTGGTTGC
RARα probe	CACTATCTCTTCAGAACTTT*TTGTCTCAAGCTTATGAA*ATGAACTC AAGGTACG
Link DNA	NH_2-TTTTTTCTCGTACGCGTACCTTGAGTTCAT
Assistant sequence	ATGAACTCAAGGTACGCGTACGAG
FAM probe	6-FAM -TGGGTCTCAATGGCTGCCTCCCC
PML/RARα Bcr1 (L) Target	CCCAACAGCAACCACGTGGCCAGTGGCGCCGGGGAGGCAGCCA TTGAGACCCAGAGCAGCAGTTCTGAAGAGATAGTGCCCAGCCCT CCCTCGCCACCCC
PML/RARα Bcr2 (V) Target	AGGTCATCAAGATGGAGTCTGAGGAGGGGAAGGAGGCAAGCCA TTGAGACCCAGAGCAGCAGTTCTGAAGAGATAGTGCCCAGCCCT CCCTCGCCACCCC
PML/RARα Bcr3 (S) Target	CGCCTGCAGGACCTCAGCTCTTGCATCACCCAGGGGAAAGCCAT TGAGACCCAGAGCAGCAGTTCTGAAGAGATAGTGCCCAGCCCT CCCTCGCCACCCC
PML DNA	CCCAACAGCAACCACGTGGCCAGTGGCGCCGGGGAGGCAGAGG AACGCGTTGTGGTGATCAGCAGCTCGGAAGACTCAGATGCCGA AAACTCGGTCTCTT
RARα DNA	CTTGAATCCTGCCAGCAGTGCCATTGAGACCCAGAGCAGC CCATTGAGACCCAGAGCAGCAGTTCTGAAGAGATAGTGCCCAG CCCTCCCTCGCCACCCC
Short target	GCGCCGGGGAGGCAGCCATTGAGACCCAGAGCAGC
SM PML/RARα Bcr1 (L) Target	CCCAACAGCAACCACGTGGCCACTGGCGCCGGGGAGGCAGCCA TTGAGACCCAGAGCAGCAGTTCTGAAGAGATAGTGCCCAGCCCT CCCTCGCCACCCC
TM PML/RARα Bcr1 (L) Target	CCCAACAGCAACCACGTGGCCGCCGGCGCCGGGGAGGCAGCCA TTGAGACCCAGAGCAGCAGTTCTGAAGAGATAGTGCCCAGCCCT CCCTCGCCACCCC

NC PML/RARα	CCCAACAGCAACCACGTGGCTGCCAGCGCCGGGGAGGCAGCCA
Bcr1 (L) Target	TTGAGACCCAGAGCAGCAGTTCTGAAGAGATAGTGCCCAGCCCT CCCTCGCCACCCC
TWM PML/RARα	CCCAACAGCAACCACGTGGCCAGTGGCGCCGGGGAGGCAGCCA
Bcr1 (L) Target	TTGAGACCCAGAGCAGCAGCTCTGTAGAGATAGTGCCCAGCCCT CCCTCGCCACCCC

[a]SM, single-base mismatched oligonucleotide; TM, three-base mismatch oligonucleotide; NC, non-complementary oligonucleotide; TWM, two mismatch oligonucleotide (The purple and underline portions represent mismatch oligonucleotides). The underline portions represent complementary sequence between RARα probe and Link DNA. The italic portions represent complementary sequence between PML probe and RARα probe. The red portions represent complementary sequence between Link DNA and Assistant sequence. The blue portions represent complementary sequence between FAM probe and exposed "L" subtype fusion gene target.

Table S2 Fluorescence intensity of 10 nM target for replicate tests at intra-assay and inter-assay

Group	Parallel tests					Average	RSD[a]	CV[b] (%)
Intra-assay	532	546	539	545	538	540	5.72	1.05
Inter-assay	529	548	535	541	537	538	7.07	1.31

[a] Relative standard deviation, [b] Coefficient of variation.

Table S3 Detection of PML/RARα fusion fragment in serum samples with salmon sperm DNA by the proposed biosensor

Sample no.	Spiking value (nM)	Assayed value (nM)	RSD[a]	CV[b] (%)	Recovery (%)
1	0.01	0.0105	6.82	5	105
2	0.1	0.0986	6.02	1.4	98.6
3	1.0	1.035	5.51	3.5	103.5

[a] Relative standard deviation, [b] Coefficient of variation.

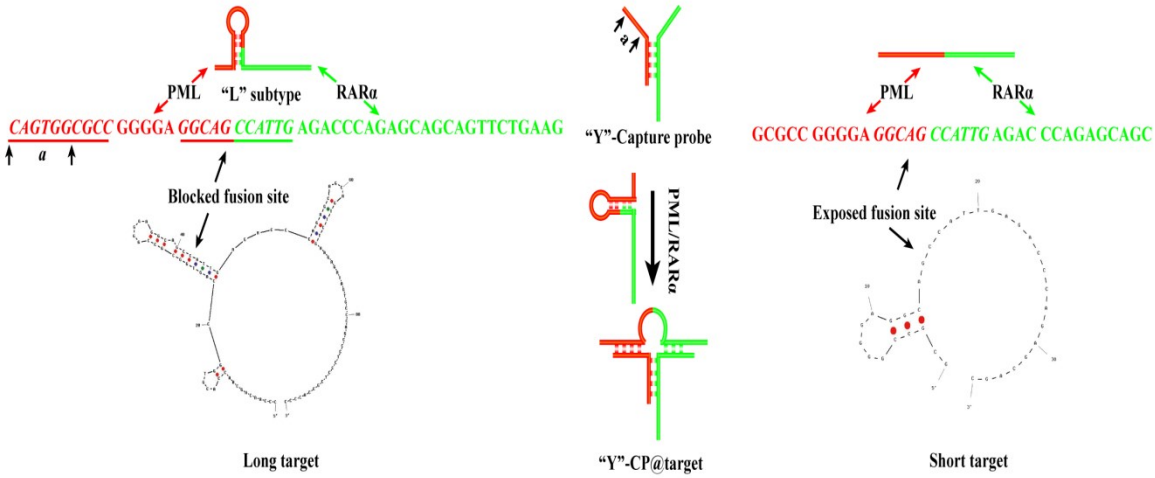


Fig. S1 The principle for "Y"-junction-nanostructure to unlock the stem loop structure of PML/RARα DNA "L" subtype.

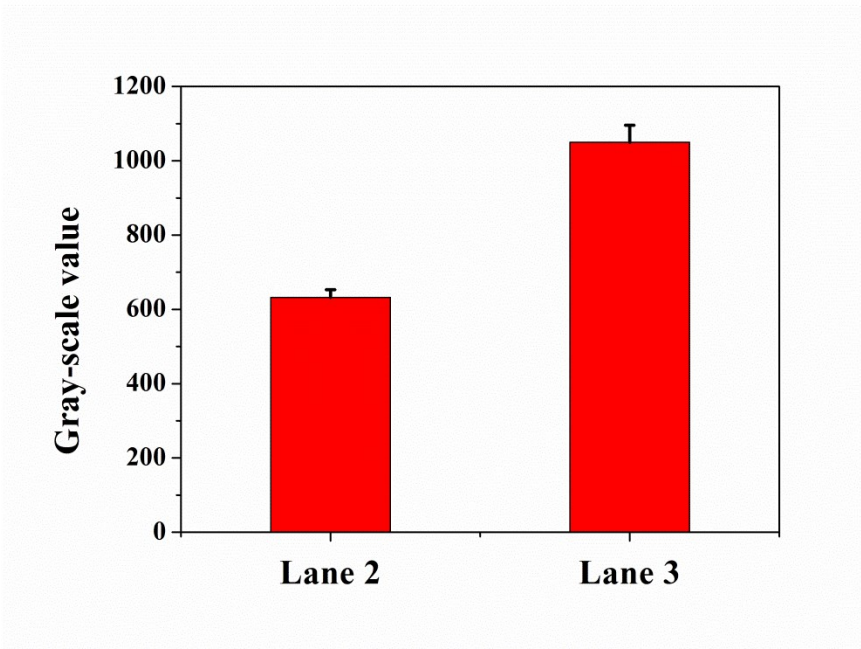


Fig. S2 Gray-scale value of electrophoresis band of "Y"-CP before (Fig. 1B, Lane 3) and after (Fig. 1B, Lane 2) specific hybridization between MBPs and "Y"-CP.

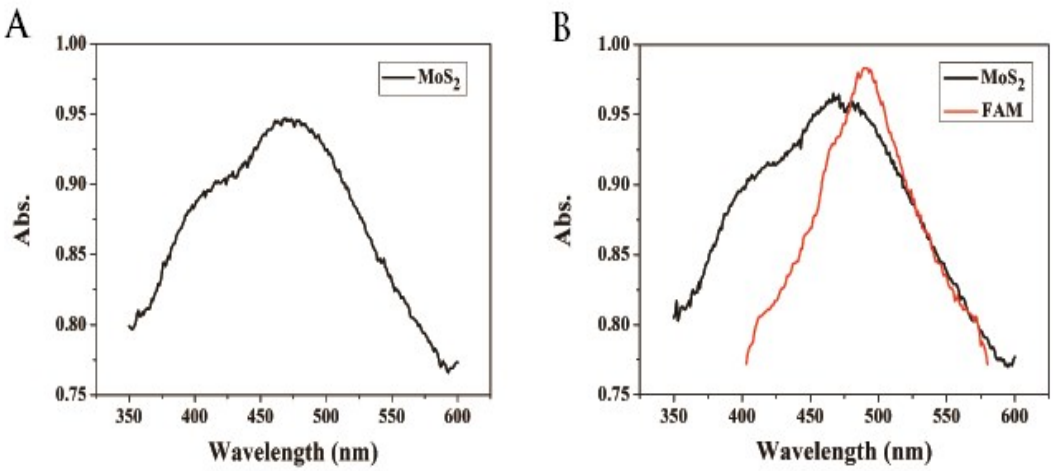


Fig. S3 (A) UV-vis spectrum of MoS_2 nanosheets before adding FAM. (B) UV-vis spectrum of MoS_2 nanosheets after adding FAM.

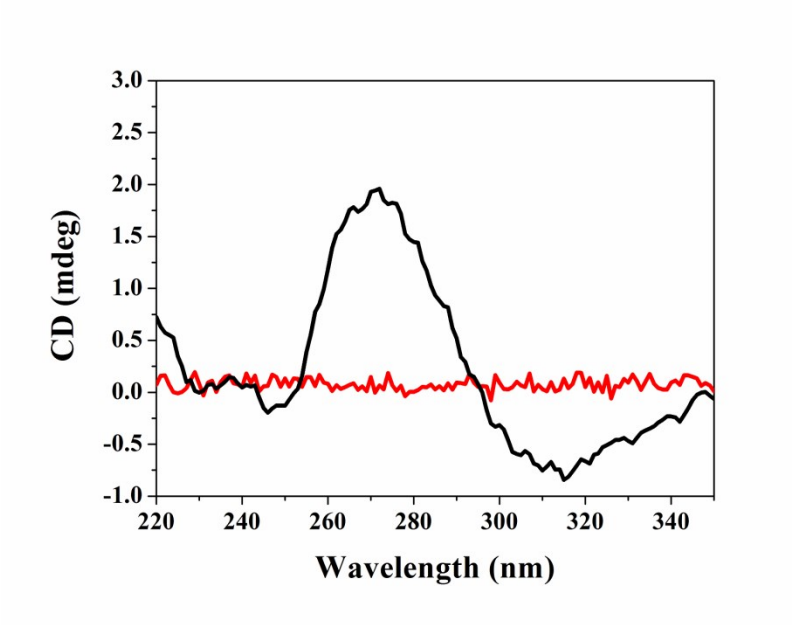


Fig. S4 CD spectra for long PML/RARα DNA with stem loop secondary structures (black curve) and short PML/RARα DNA with exposed fusion gene (red curve).

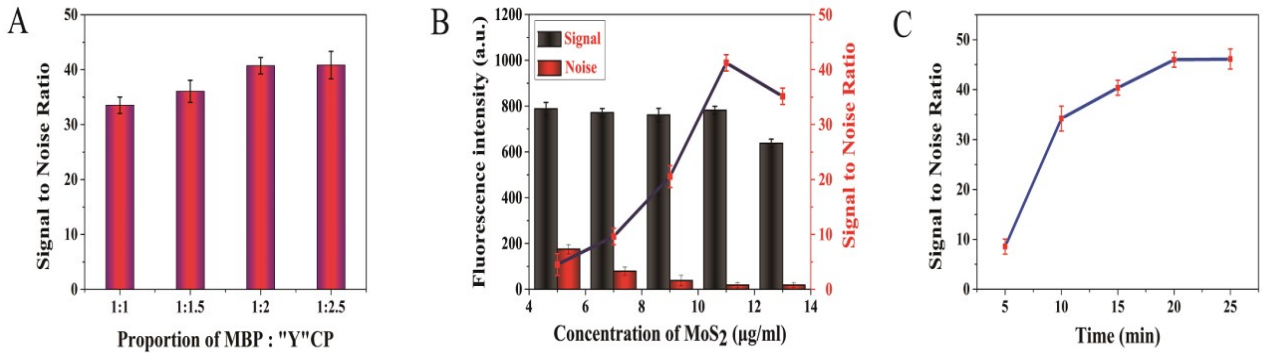


Fig. S5 Optimization of experiment conditions. (A) Evaluation of proportion of MBP: "Y"-CP; (B) Evaluation of MoS_2 concentration; (C) Evaluation of hybridization time between FAM-probe and "Y"-CP@long DNA target. Error bar represents the standard deviation (n = 3).

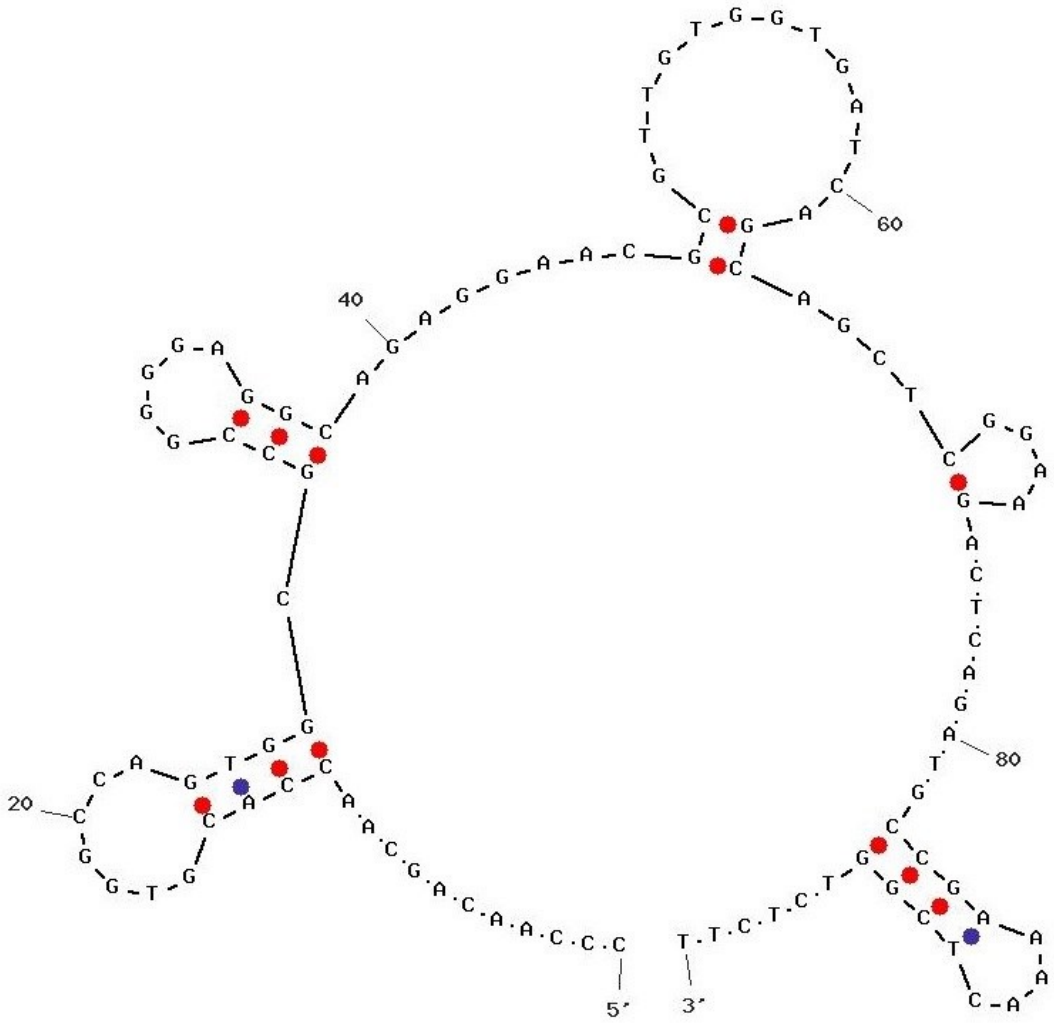


Fig. S6 The secondary structures of non-targets: PML.

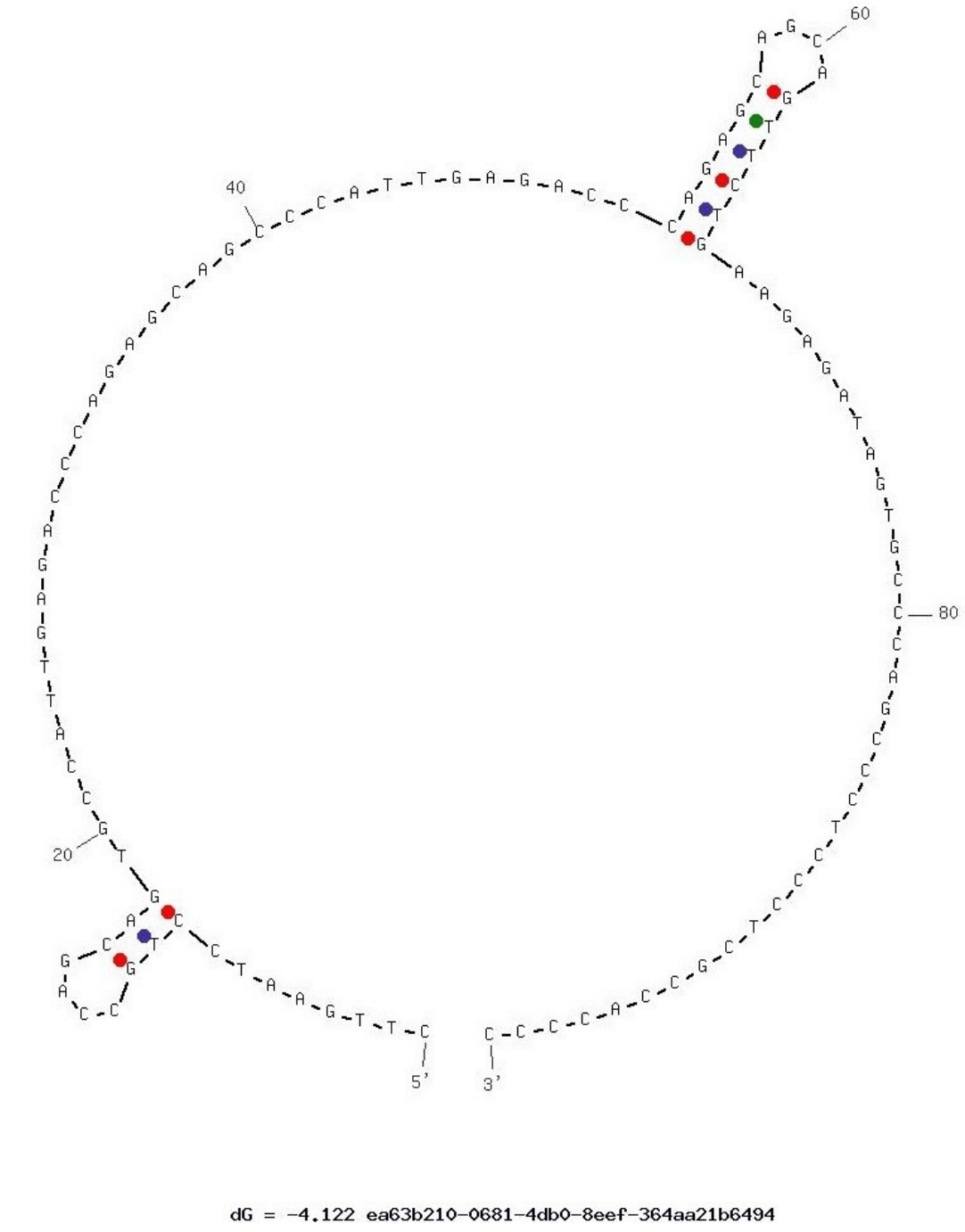
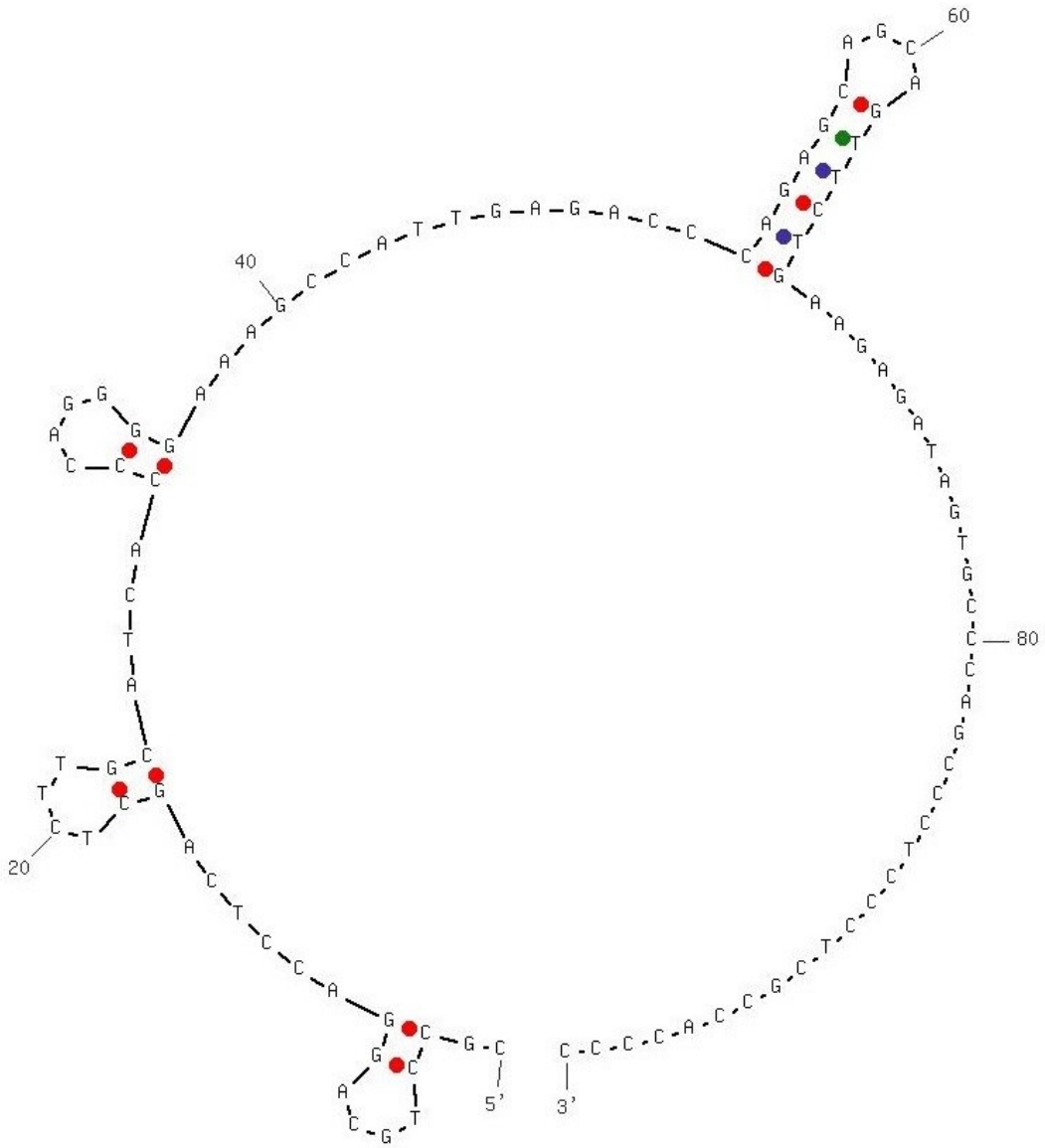


Fig. S7 The secondary structures of non-targets: RARα.



dG = -6.632 470284c8-55e0-4fe4-8e93-59f3b043a8f9

Fig. S8 The secondary structures of non-targets: “S” subtype.

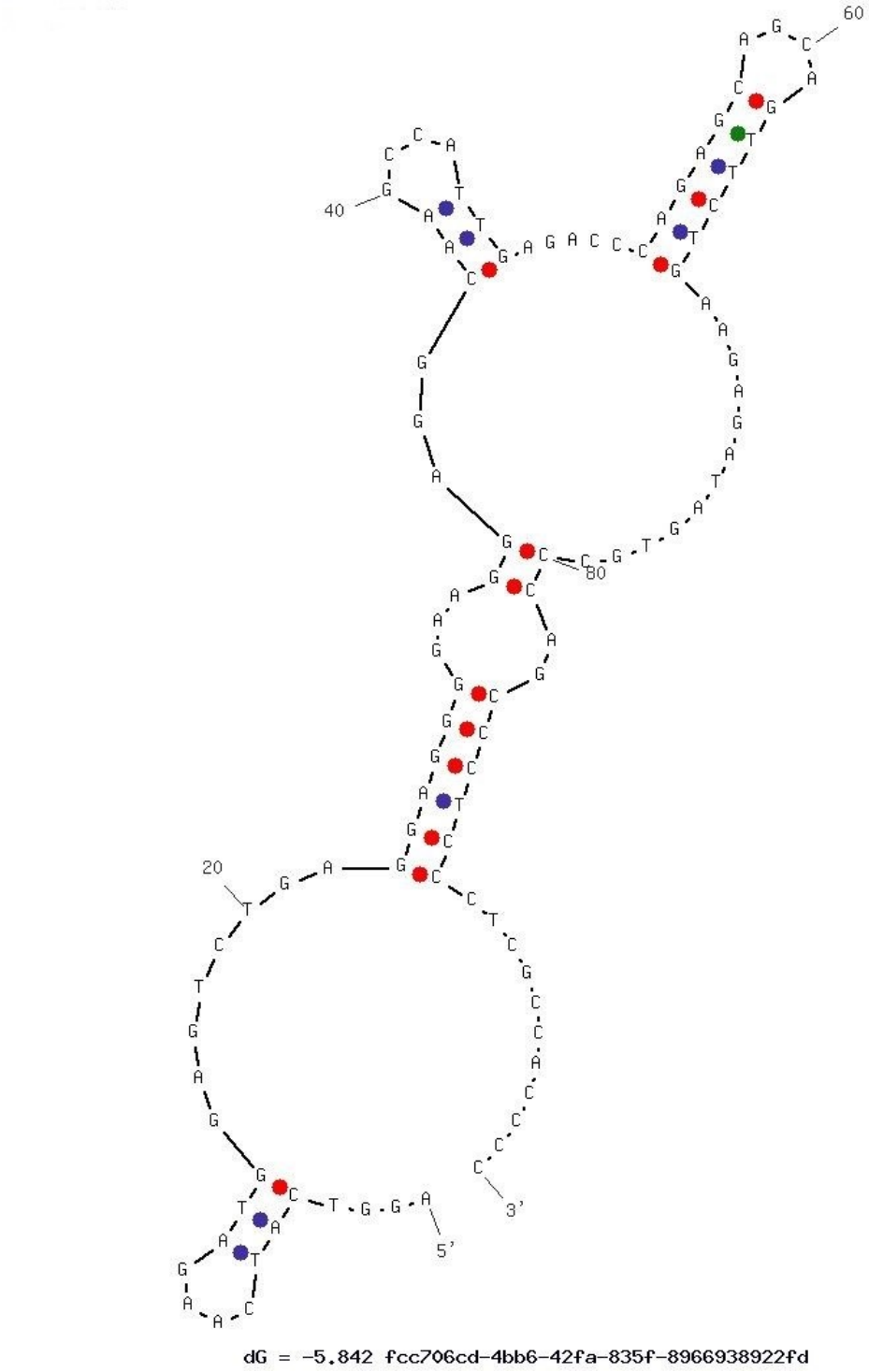


Fig. S9 The secondary structures of non-targets: "V" subtype.

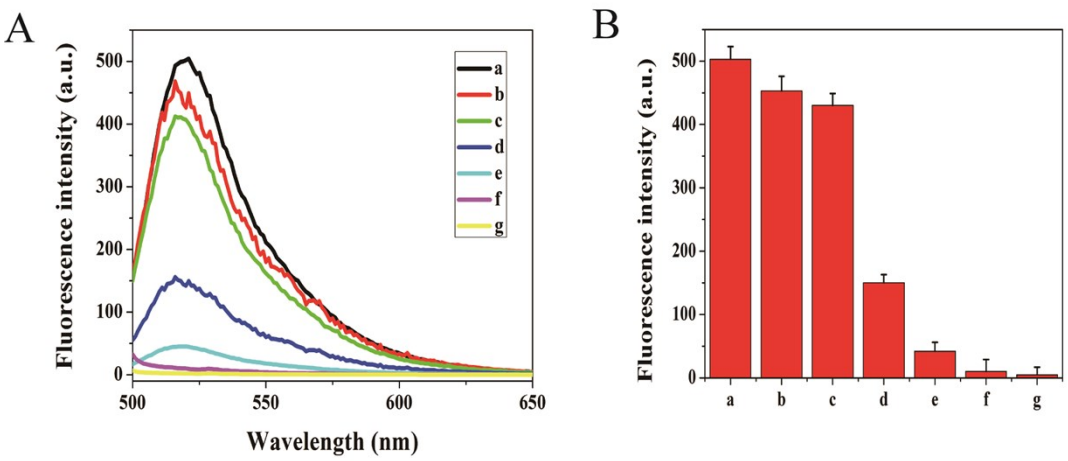


Fig. S10 Fluorescence emission spectra (A) and fluorescence response signals (B) for selectivity and stability of PML/RARα detection against short target (a), PML/RARα DNA "L" subtype (b), two-bases mismatch in RARα portion (c), single-base mismatch in PML a portion (d), three-bases mismatch in PML a portion (e), non-complementary in PML a portion (f), and blank (g). Error bar represents the standard deviation (n = 3).