Electronic Supplementary Information

Toehold-regulated Competitive Assembly to Accelerate Kinetics of Graphene Oxide-based Biosensors

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Table S1. All the DNA sequences used in this work

Oligonucleotides			Sequences (5'→3')
Oligonucleotides used in the principle of toehold-regulated competitive assembly (A)			
Strategy A	Reporter-1		AGG AA GGC AAC TCA-FAM
	Correction strand-1		AGG AA GGC AAC TCA
	Target-1		TGA GTT GCC TT CCT
	BT (blocker on target-1)		GGC AAC TCA
	BT/TB (blocker on target-1)		GGC AAC TCA GTT
	Evaluation of length of toehold in target/blocker in strategy A-b		
	Toe-3		AAG GCA ACT CA GTT
	Toe-4		AG GCA ACT CA GTT
	Toe-5		GGC AAC TCA GTT
	Toe-6		GCA ACT CA GTT
Strategy B	Reporter-2		**AAG GCA GTA CGG AA GGC AAC TCA-FAM**
	Correction strand-2		AAG GCA GTA CGG AA GGC AAC TCA
	BR (blocker on reporter -2)		CTT CCG TAC
	Target-2		TGA GTT GCC TTC CGT ACT GCC TT
	BT (blocker on target-2)		GGC AAC TCA
	BT/TB (blocker on target-2)		GGC AAC TCA GTT
	Evaluation of length of toehold in target/blocker in strategy B-c		
	Toe-11		GAA GGC AAC TCA GTT
	Toe-12		AA GGC AAC TCA GTT
	Toe-13		AG GCA ACT CA GTT
	Toe-14		G GCA ACT CA GTT
	Toe-15		GCA ACT CA GTT
	Optimism of TR and BR on reporter-2 in strategy-B		
	Blocker with 3	T3-B8	CG TAC TGC
		T3-B9	CCG TAC TGC

Strategy B	toehold to reporter-2 (Toe-3)	T3-B10	T CCG TAC TGC
		T3-B11	TT CCG TAC TGC
		T3-B12	CTT CCG TAC TGC
	Blocker with 4 toehold to reporter -2(Toe-4)	T4-B8	CCG TAC TG
		T4-B9	T CCG TAC TG
		T4-B10	TT CCG TAC TG
		T4-B11	CTT CCG TAC TG
		T4-B12	C CTT CCG TAC TG
	Blocker with 5 toehold to reporter -2(Toe-5)	T5-B8	T CCG TAC T
		T5-B9	TT CCG TAC T
		T5-B10	CTT CCG TAC T
		T5-B11	C CTT CCG TAC T
		T5-B12	GC CTT CCG TAC T
	Blocker with 6 toehold to reporter -2(Toe-6)	T6-B8	TT CCG TAC
		T6-B9	CTT CCG TAC
		T6-B10	C CTT CCG TAC
		T6-B11	GC CTT CCG TAC
	Blocker with 7 toehold to reporter -2(Toe-7)	T7-B8	CTT CCG TA
		T7-B9	CCT TCC GTA
		T7-B10	G CCT TCC GTA
	Blocker with 8 toehold to reporter -2(Toe-8)	T8-B8	CCTT CCG T
		T8-B9	G CCT TCC GT
Oligonucleotides used in thrombin detection			
Strategy A-proteins	29-mer aptamer reporter		AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-FAM
Strategy B-proteins	Blocker of Apatamer		**TGC CCT ACC**
	Blocked aptamer reporter		**CC ATC CCG T** AGT CCG T**GG TAG GGC A**GG TTG GGG TGA CT-FAM

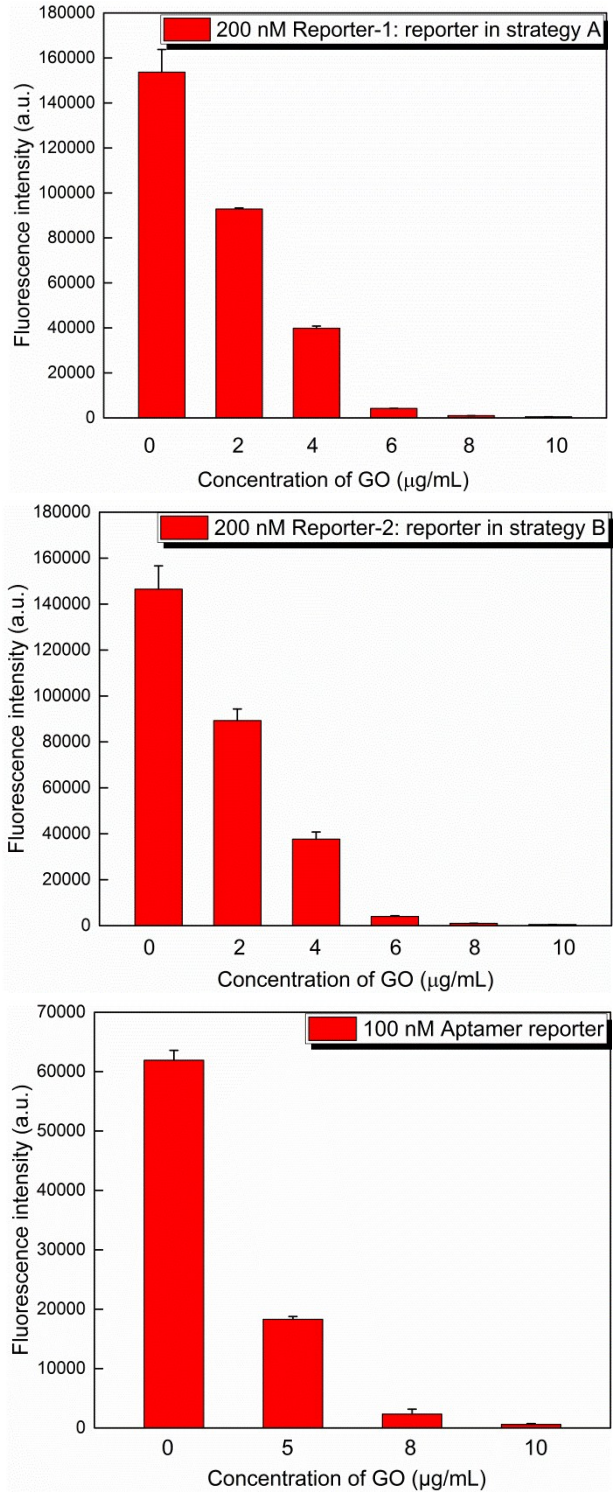

Fig. S1 Investigation of GO concentration for quenching fluorophore reporter-1 in strategy A, reporter-2 in strategy B and aptamer reporter. 10 μg/mL GO for strategy A and 8 μg/mL GO for strategy B. Error bars showed the standard deviations of three paralleled samples. GO and reporters were incubated at room temperature for an hour, and then transferred to the 96-well microplate for endpoint fluorescence measurement. The excitation and the emission wavelengths are 485 nm and 520 nm, respectively.

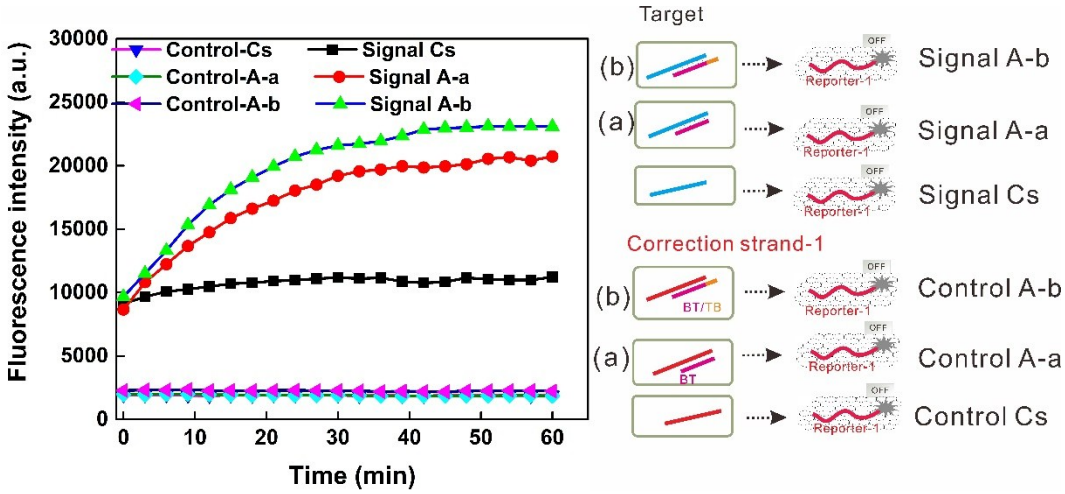

Fig.S2 The effect of toehold format on competitive assembly (strategy A). Control-Cs contained GO (10 μg/mL), reporter-1 (200 nM) and correction strand-1 (200 nM); Control-A-a contained GO (10 μg/mL), reporter-1 (200 nM) and correction strand-1-BT (200 nM); Control-A-b contained GO (10 μg/mL), reporter-1 (200 nM) and correction strand-1-BT/TB (200 nM); Signal-Cs contained GO (10 μg/mL), reporter-1 (200 nM) and Target-1 (200 nM); Signal-A-a contained GO (10 μg/mL), reporter-1 (200 nM) and Target-1-BT (200 nM); Signal-A-b contained GO (10 μg/mL), reporter-1 (200 nM) and Target-1-BT/TB (200 nM); They were transferred to the 96-well microplate at RT for one hour fluorescence measurement. The excitation and the emission wavelengths are 485 nm and 520 nm, respectively.

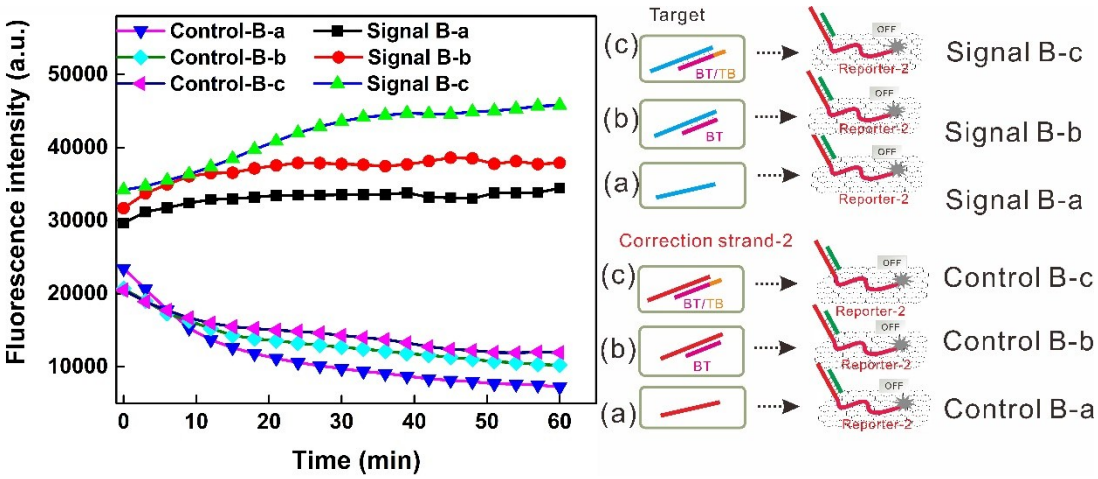

Fig.S3 The effect of dual-toehold format on competitive assembly (strategy B). Control-B-a contained GO (10 μg/mL), blocked reporter-2 (200 nM) and correction strand-2 (200 nM); Control-B-b contained GO (10 μg/mL), blocked reporter-2 (200 nM) and correction strand-2-BT (200 nM); Control-B-c contained GO (10 μg/mL), reporter-1 (200 nM) and correction strand-2-BT/TB (200 nM); Signal-B-a contained GO (10 μg/mL), blocked reporter-2 (200 nM) and Target-2 (200 nM); Signal-B-b contained GO (10 μg/mL), blocked reporter-2 (200 nM) and Target-2-BT (200 nM); Signal-B-c contained GO (10 μg/mL), blocked reporter-2 (200 nM) and Target-2-BT/TB (200 nM); They were transferred to the 96-well microplate at RT for one hour fluorescence measurement. The excitation and the emission wavelengths are 485 nm and 520 nm, respectively.

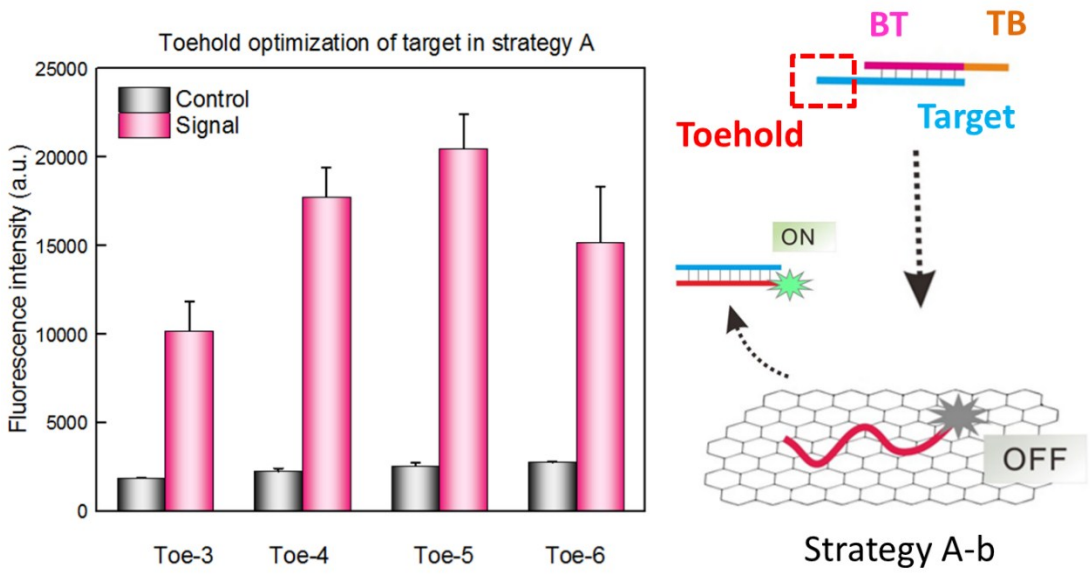

Fig.S4 The effect of length of toehold (TT) in target/blocker in strategy A-b. The mixtures contained 10 μg/mL GO, 200 nM reporter-1 and 200 nM target/blocker with different toeholds. Then they were incubated at room temperature for an hour, and then transferred to the 96-well microplates for endpoint fluorescence measurement. The optimal one was toehold-5 (Toe-5). All the data were recoded with the excitation and emission wavelength of 485 nm and 520 nm, respectively, and from at least three independent experiments.

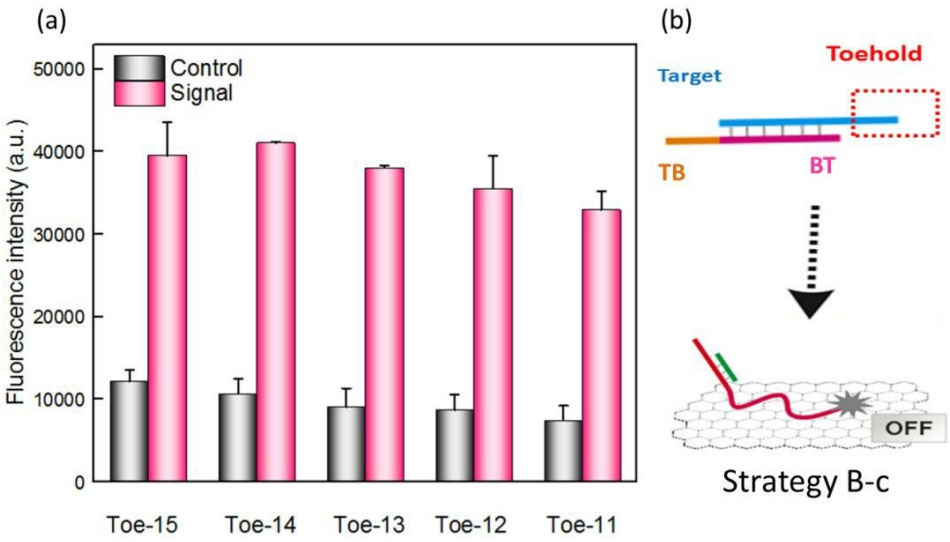

Fig.S5 The effect of length of toehold (TT) in target/blocker in strategy B-c. The mixtures contained 10 μg/mL GO, 200 nM reporter-2 and 200 nM target/blocker with different toeholds. Then they were incubated at room temperature for an hour, and then transferred to the 96-well microplates for endpoint fluorescence measurement. The concentration of target-c was 200 nM. The optimal one was toehold-14 (Toe-14). All the data were recoded with the excitation and emission wavelength of 485 nm and 520 nm, respectively, and from at least three independent experiments.

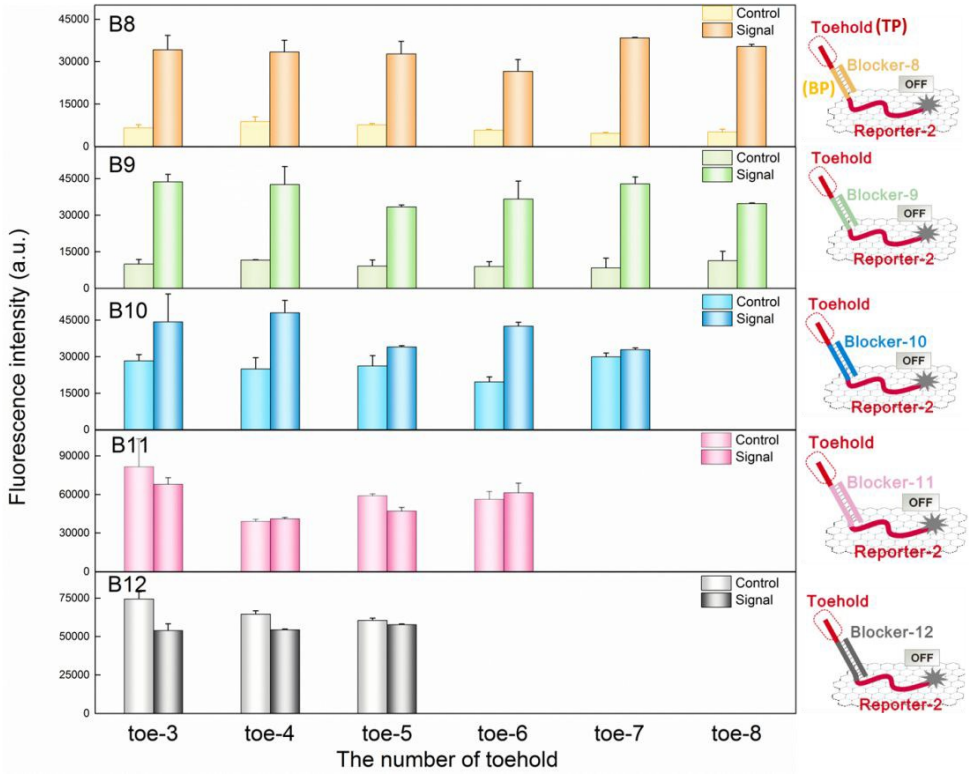

Fig.S6 Evaluation effect of length of toehold in reporter/blocker in strategy B. The mixture contained 10 μg/mL GO, 200 nM reporter-2 with different length of blocker (BR) and toehold (TR), and 200 nM Target-2-BT/TB. They were incubated at room temperature for an hour, and then transferred to the 96-well microplates for endpoint fluorescence measurement. The optimal one was blocker-9/toehold-7 (T7-B9). All the data were recoded with the excitation and emission wavelength of 485 nm and 520 nm, respectively, and from at least three independent experiments.

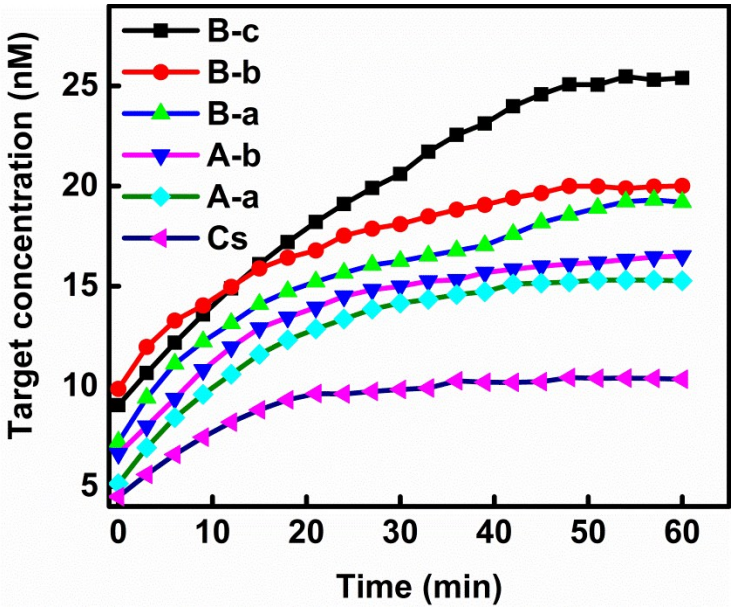

Fig.S7 Kinetics of C/t curves of six strategies: Cs, A-a, A-b, B-a, B-b, B-c.
Target concentration (C) was set as a function of time (t). According to the kinetics equation： $[target\ bound]_t = [target\ bound]_{max} * e^{kobs*t}$, the initial targets concentrations were 1 nM, when t=40 min, the target concentration of each strategy were 24.0 nM, 19.4 nM, 17.5 nM, 15.8 nM, 15.1 nM, 10.2 nM, so according to the kinetics equation, the calculated k_{obs} values of strategy Cs, A-a, A-b, B-a, B-b, B-c were 0.058 min-1, 0.067 min^{-1}, 0.069 min^{-1}, 0.071 min^{-1}, 0.074 min^{-1}, 0.080 min^{-1}, respectively.

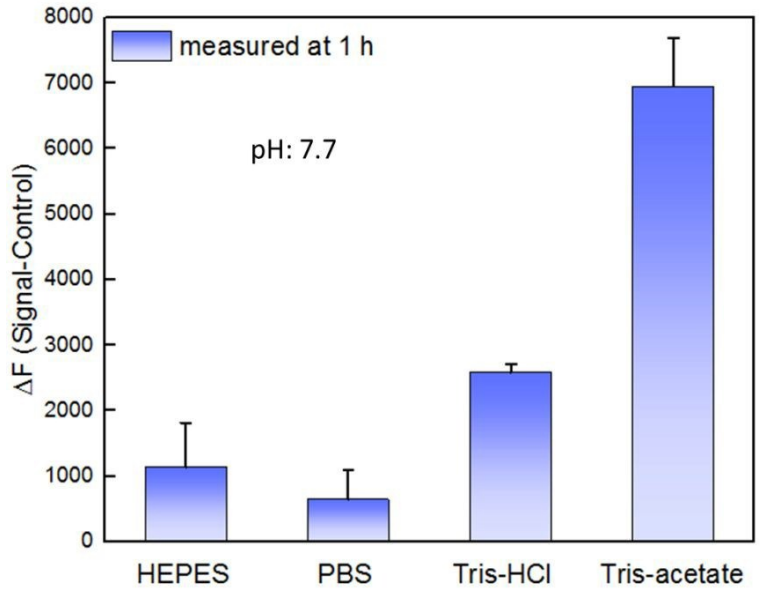

Fig.S8 Evaluations of performance of toehold-accelerated sensor based thrombin detection in four kinds of buffers at pH 7.7. The mixture with different buffers were incubated at room temperature for an hour, and then transferred to the 96-well microplates for endpoint fluorescence measurement. The thrombin concentration was 100 nM. All the data were recoded with the excitation and emission wavelength of 485 nm and 520 nm, respectively, and from at least three independent experiments.

Table S2 GO-based biosensor for nucleic acid and protein sensing

Type	Sensitivity	Detction time	Selectivity (target: interferents), Discrimination fator[1]	Amplification strategy	Ref.
premixing	≈10 nM for DNA ≈ 2 nM for thrombin	Slow (0.5 h)	Thrombin (1:1), 4	No amplification	Angew. Chem. Int. Ed. 2009 , 48 , 4785 .
premixing	≈1 nM for DNA	Slow (0.6 h)	Cysteine (1:1), 4	No amplification	Biosens. Bioelectron. 2011, 26 (7), 3260-3265.
premixing	22.8 pM for thrombin	Slow (2.7 h)	Thrombin (1:1), 4	CHA & enzyme amplification	ACS Sensors 2018, 3 (11), 2423-2431.
postmixing	100 pM for miRNA	Slow (4.5 h)	miRNA (1:1), 2.5	HCR amplification	ACS Appl. Mater. Interfaces 2012, 4 (12), 6450-3.
postmixing	100 pM for DNA	Rapid (minutes)	Adenine (1:1), 3.5	No amplification	Adv. Funct. Mater. 2010 , 20 , 453-459 .
premixing	≈1 nM for DNA ≈10 nM for thrombin	Rapid (minutes)	Thrombin (1:20), 3	No amplification	This work

[1] Discrimination factor equals to the signal intensity ratio of target to interferents