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Electronic Supplementary Information

Integrating dye-intercalated DNA dendrimers with electrospun nanofibers: a new fluorescent sensing platform for nucleic acids, proteins, and cells

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Materials and reagents

Poly(styrene-co-maleic acid) (PSMA) (M_w=65000) was purchased from Sigma-Aldrich (St. Louis, MO, USA). N, N-dimethylformamide (DMF) was purchased from Beijing Chemical Work, (Beijing, China). N-(3-dimethyl-aminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) was obtained from J&K Scientific (Beijing, China). N-hydroxysuccinimide (NHS) was ordered from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). HPLC-purified DNA oligonucleotides were ordered from Sangon Inc. (Shanghai, China), and their sequences were listed in Table S1−S3. Thrombin (Thb) was obtained from Enzyme Research (South Bend, IN, USA). Other interfering proteins including human serum albumin (HSA), lysozyme (Lzm), hemoglobin (Hb), and fibrinogen (Fib) were ordered from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Solarbio (Beijing, China). Phosphate buffer saline (PBS) (150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH=7.4) was used as the buffer solution. All other reagents were of analytical grade, and deionized water was used in all experiments.

Characterization

The PSMA electrospun nanofibers were fabricated using a set of homemade electrospinning setup, which contained a high voltage supply (Beigao, Beijing, China), a syringe pump (Lion, Zhejiang, China) and a grounded rotary collector.[1] The average diameter of the G_3 DNA dendrimer was measured using an ALV/DLS/SLS-5022F laser light scattering spectrometer (ALV/Laser Vertriebsgesellschaft m. b. H, German). The morphologies of the G_3 and NF-capture/target/reporter-G_3SG sandwich-type nanocomplex were characterized using an S-4800 scanning electron microscope (Hitachi, Japan) and an SPA400 atomic force microscopy (Seiko Instruments, Japan). The fluorescent images were observed by an A1Rsi laser scanning confocal microscopy (Nikon, Japan). Fluorescence spectra were determined using an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). UV-vis absorption spectra were recorded on a U-3010 spectrophotometer (Hitachi, Tokyo, Japan). Fluorescence lifetime was measured using an FLS920 steady state and lifetime fluorescence spectrometer (Edinburgh instrument, UK).

Agarose gel electrophoresis experiments

The 1.5 % agarose gels containing 0.05 μL SYBG I dye per milliliter of gel volume were prepared by using 1×TAE buffer. 5 μL DNA sample was mixed with 1 μL 6×glycerol loading buffer (Sangon, Shanghai, China) and loaded into the gel. The gel electrophoresis was conducted at a constant voltage of 110 V for 1 h and scanned by a Tanon 1600 imager (Shanghai, China).

Fluorescence quantum yield measurement

To measure the quantum yield (Φ_F (G_3SG)) of the G_3SG, the solution of the G_3SG was diluted to an absorbance of 0.05. The absorbance of G_3SG solution at 488 nm and theintegrate areas of the fluorescence-corrected spectra of G_3SG were recorded.The relative fluorescence quantum yield of the G_3SG was obtained using fluorescein in 0.1 M NaOH (Φ_F (fluorescein) = 0.92) as the standard.

Quantification of capture probe densities on the sensing membranes

The NF-capture membrane of 1.4×2.5 cm^2 was first incubated with 400 μL, 2 μM solution of FAM-labeled complementary DNA(ACTTCATCCACGT-FAM) overnight to allow fully hybridization. After washing off the non-specific adsorption

with PBS buffer, the membrane was incubatedwith100 μL EtOH to dehybridize the FAM-labeled DNA. The total supernatant containing the FAM-labeled DNA was diluted to 2 mL with PBS and its fluorescence intensity was recorded. A standard curve of the FAM-labeled DNA from 0.5 to 50 nM was also prepared using the same PBS buffer. The capture probe density on the sensing membrane was calculated based on the amount of the FAM-labeled DNA and the surface area of the sensing membrane.

Spike recovery test

In order to evaluate the feasibility of the proposed sensing platform in a real physiological media, the targets, 1 nM β-thalassemia gene fragment and thrombin, and 1000 cells/mL HeLa cells, were respectively added into the diluted fetal bovine serum (FBS). The fluorescence increment of the spiked and unspiked samples were recorded as ΔF (target+FBS) and ΔF(FBS). And the fluorescence signal of the spiked target in FBS was obtained as follow:

$$\Delta F(target) = \Delta F(target + FBS) - \Delta F(FBS)$$

The detected target concentration in the spiked FBS (c(detected)) was calculated based on ΔF(target) and the corresponding linear regression equation. And the spike recovery was calculated based on c(detected) and c(added):

$$Recovery = \frac{n(detected)}{n(added)} \times 100\% = \frac{c(detected)}{c(added)} \times 100\%$$

Table S1. DNA oligonucleotide sequences for the step-by-step assembly of the DNA dendrimer backbones.

Name	Sequences (5' to 3')[a]	
	Sticky ends	Common sequences
Y_{0a}_strand	TGTCATATTCGTA	GTTCTGTCTCGCTTAAGTTTAACATG
Y_{1a}_strand	CGCTTATCTGGGA	
Y_{2a}_strand	ACCACTCCACCTC	
Y_{3a}_strand	GTTCTGTGTAAAT	
Y_{4a}_strand	TCACCTCTACTAC	
Y_{0b}_strand	TGTCATATTCGTA	CATGTTAAACTTACTATCTCTACTCT
Y_{1b}_strand	CGCTTATCTGGGA	
Y_{2b}_strand	ACCACTCCACCTC	
Y_{3b}_strand	GTTCTGTGTAAAT	
Y_{4b}_strand	TCACCTCTACTAC	
Y_{0c}_strand	TGTCATATTCGTA	AGAGTAGAGATAGAGCGAGACAGAAC
Y_{1c}_strand	TACGAATATGACA	
Y_{2c}_strand	TCCCAGATAAGCG	
Y_{3c}_strand	GAGGTGGAGTGGT	
Y_{4c}_strand	ATTTACACAGAAC	

[a]The sticky ends of each generation of DNA dendrimers are shown in color: G_0: green, G_1: yellow, G_2: blue, G_3: red, and G_4: purple.

Table S2. The capture and reporter DNA sequences for the sensing of β-thalassemia gene fragment, thrombin, and HeLa cells.

Target	Probe	Sequences (5' to 3')[a]
β-thalassemia gene fragment	Capture	**ACGTGGATGAAGT**TTTTTT-NH_2
	Reporter	ATTTACACAGAAC**AAGGGGCAAGGT**
	Target	ACTTCATCCACGTTCACCTTGCCCCTT
	1-Mismatch	ACTTCATCCACGTTCACCTAGCCCCTT
	2-Mismatch	ACTTCATCCACGTTCACCTACCCCCTT
	3-Mismatch	ACTTCATCCACGTTCACCTACGCCCTT
	Random	ACTGACTGACTGACTGACTGACTGACT
Thrombin	Capture (TBA15)	NH_2-TTTTTTTTTT**GGTTGGTGTGGTTGG**
	Reporter (TBA29)	ATTTACACAGAAC**AGTCCGTGGTAGGGCAGGTGGGGGTGACT**
HeLa cells	Capture (Sgc8 aptamer)	NH_2-TTTTTT**ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA**
	Reporter (Sgc8 aptamer)	ATTTACACAGAAC**ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA**

[a]The single-stranded domains to hybridize with the sticky ends of G_3 are marked in color. The functional domains to bind with the targets are marked in bold type. The mismatched bases in the mutant target sequences are underlined.

Table S3. Different reporter DNA sequences for evaluating the effect of fluorescent signal probes on the sensitivity of the sensing platforms.

Probe	Sequences (5' to 3')[a]
Reporter$_0$	TACGAATATGACA**AAGGGGCAAGGT**
Reporter$_1$	TCCCAGATAAGCG**AAGGGGCAAGGT**
Reporter$_2$	GAGGTGGAGTGGT**AAGGGGCAAGGT**
Reporter$_3$	ATTTACACAGAAC**AAGGGGCAAGGT**
Reporter$_4$	GTAGTAGAGGTGA**AAGGGGCAAGGT**
Reporter-FAM	FAM-**AAGGGGCAAGGT**

[a]The single-stranded domains to hybridize with the sticky ends of the DNA dendrimers are marked in color. The functional domains to bind with the targets are marked in bold type.

Table S4. Comparison between the proposed fluorescent sensing platform with other non-enzymatic and enzymatic fluorescent approaches for nucleic acid detection.

Assay format	Label	LOD	Referenc e
Non-enzymatic approach			
Sandwich hybridization assay on electrospun nanofibers	Dye-intercalated DNA dendrimer	20 pM	This work
ThT based molecular beacon	Thioflavin T	6.5 nM	2
Sandwich hybridization assay	Fluorescent magnetic liposomes	35 pM	3
Photo induced electron transfer	DNA/Ag fluorescent nanoclusters	0.6 nM	4
Target-induced desorption	DNA-stabilized Ag nanoclusters	0.5 nM	5
Molecular beacon	ZnSe quantum dot	10 pM	6
De novo fluorophore synthesis	Stilbene	40 pM	7
Bio-barcode assay	AgNPs enhanced fluorescence	1 pM	8
Fluorescence quenching by MoS_2 nanosheet	FAM	500 pM	9
Graphene oxide quenched molecular beacon	FAM and Cy5	2 nM	10
Enzymatic approach			
Autocatalytic DNAzyme-Mediated Process	FAM	1 pM	11
Exonuclease-assisted autocatalytic target recycling	FAM, TAMRA, and Cy 5	20 pM	12
Exonuclease III-aided target recycling	CAL Fluor Red 610	10 pM	13
Zn^{2+}-ligation DNAzyme driven cascades	Cy3 and Cy5	20 pM	14
Endonuclease IV and lambda exonuclease coupling system	FAM	20 pM	15
DNA cross-triggered cascading circuit	SYBR Green I	100 fM	16

Table S5. The LODs of different methods for β-thalassemia gene fragment, thrombin, and HeLa cells detection.

Assay format	Detection technique	Use of enzyme	LOD	Reference
β-thalassemia gene fragment detection				
Multiplexed bead-based mesofluidic system	Fluorescence	No	10 pM	17
DNA ligase reaction and biocatalyzed deposition	Quartz crystal microbalance	Yes	0.1 nM	18
DNA ligase-mediated AuNP assembly	Colormetric	Yes	121 pM	19
Rolling circle amplification and AuNP aggregates	Colormetric	Yes	70 fM	20
Polymerization-induced enzymatic amplification	Electrochemical	Yes	0.01 pM	21
Integration of DNA dendrimers with electrospun nanofibers	Fluorescence	No	20 pM	This work
Thrombin detection				
Aptamer-based label-free fluorescing molecular switch	Fluorescence	No	2.8 nM	22
Aptamer-based unmodified AuNP probes	Colorimetric	No	0.83 nM	23
Nanocomposite of graphene and plasma polymerized allylamine	Quartz crystal microbalance	No	182 pM	24
Iodide-induced ligand displacement	Fluorescence	No	89 pM	25
Exonuclease-assisted amplification	Electrochemical	Yes	0.9 pM	26
Aptamer-based rolling circle amplification and nanoparticle signal enchancment	Surface plasmon resonance	Yes	0.78 aM	27
Integration of DNA dendrimers with electrospun nanofibers	Fluorescence	No	42 pM	This work
HeLa cells detection				
IrO_2-hemin-TiO_2 nanowire arrays	Photoelectrochemical		8000 cells/mL	28
Aggregation of primer-modified AuNPs	Colorimetric		1000 cells/mL	29
Enzyme-linked immunoassay on $PDCN_X$ nanotubes	Electrochemical		500 cells/mL	30
Carbon nanodot@Ag and graphene for dual signal amplification	Electrochemiluminescent		10 cells/mL	31
Integration of DNA dendrimers with electrospun nanofibers	Fluorescence		1500 cells/mL	This work

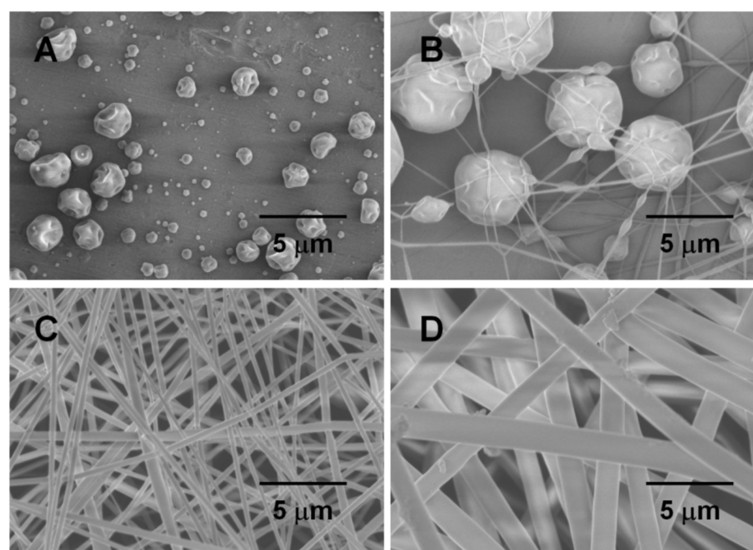

Fig. S1 SEM images of the electrospun products obtained from PSMA-DMF solution of different weigh percentages (*w*/*w*). (A) 10%, (B) 20%, (C) 40%, and (D) 50%. The 40% PSMA-DMF was used as the electrospinning solution.

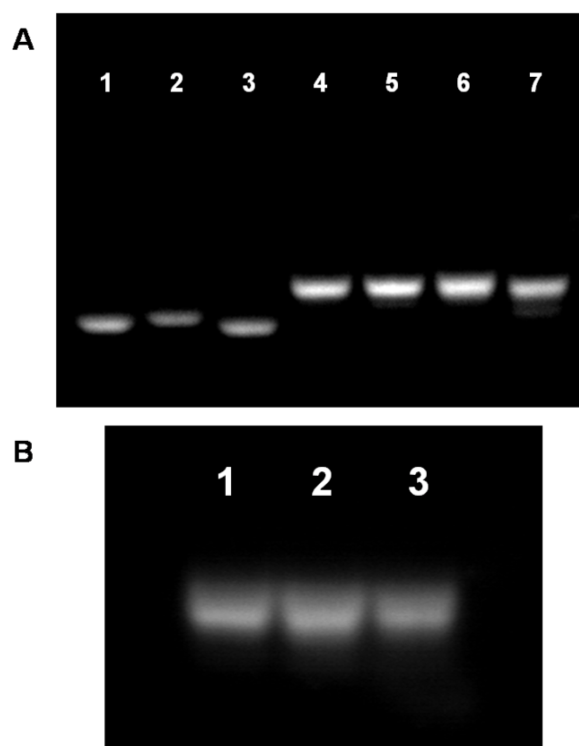

Fig. S2 Agarose gel electrophoresis showing (A) the formation of Y-DNA from three single strands. Lane 1: Y_{0a}, lane 2: Y_{0b}, lane 3: Y_{0c}, lane 4: Y_0, lane 5: Y_1, lane 6: Y_2, andlane 7: Y_3. (B) Stability of the G_3 DNA dendrimer. Lane 1: the as-prepared G_3, lane 2: G_3 kept in 4 ^{o}C for 1 week, and lane 3: G_3 kept in 4 ^{o}C for 3 weeks.

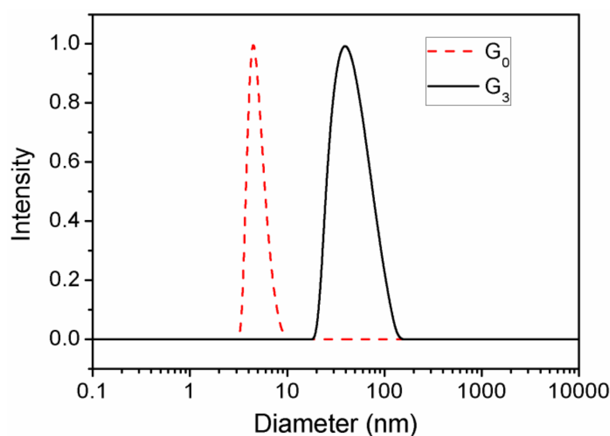

Fig. S3 Dynamic light scattering data of the G_0 and G_3 DNA dendrimers. The average diameters of G_0 and G_3 are 4.8±0.8 nm and 49±17 nm, respectively, demonstrating the growing size of the DNA dendrimers.

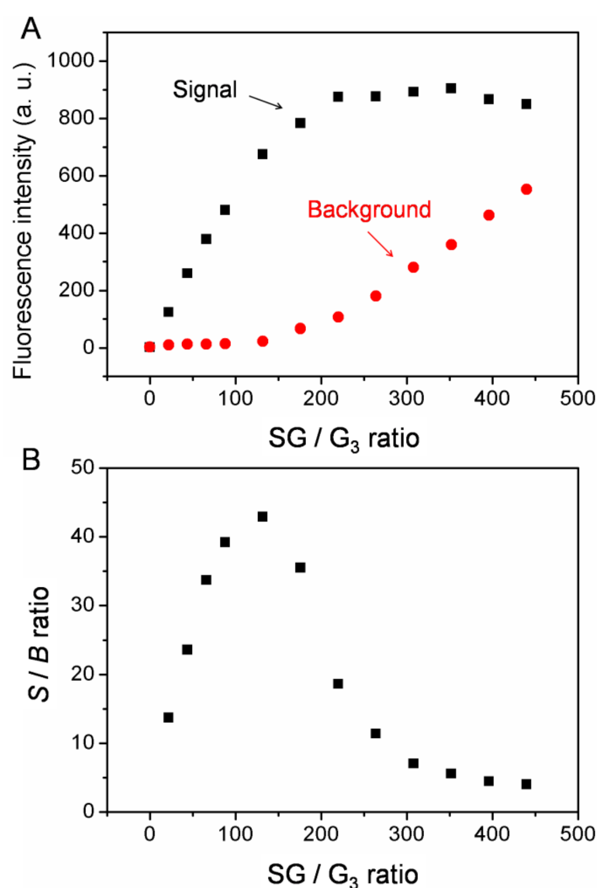

Fig. S4 (A) Dependence of fluorescence intensities on the SG/G_3 ratio. F_{signal} is the fluorescence generated by the SG molecules intercalated in the G_3 DNA dendrimer, and $F_{background}$ is the fluorescence generated by the excess SG. (B) Dependence of signal-to-background ratio ($F_{signal}/F_{background}$) to the SG/$G_3$ ratio. The $F_{signal}/F_{background}$ ratio reaches maximum at the SG/G_3 ratio of 132, corresponding to 132 SG dyemolecules intercalatedinto each G_3 DNA dendrimer.

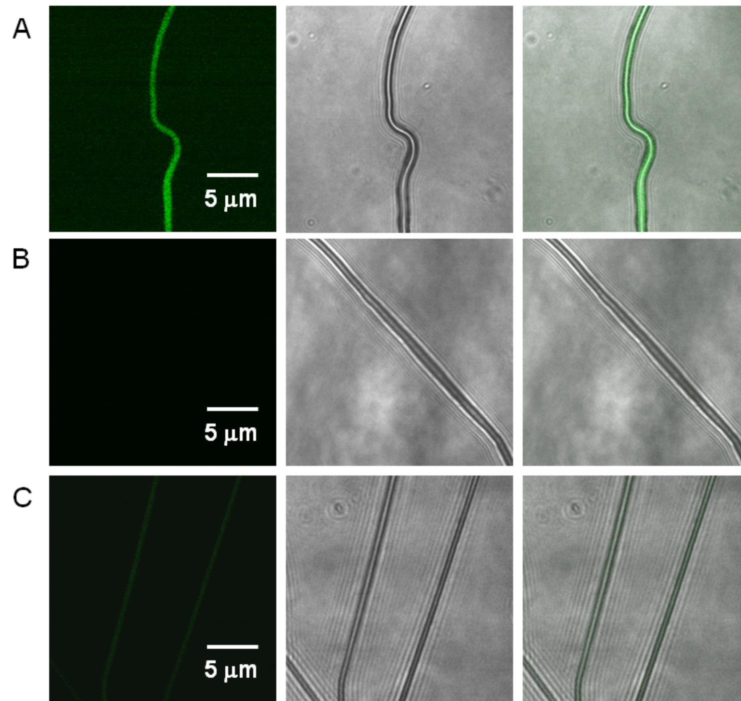

Fig. S5 Confocal fluorescence microscopy images of (A) NFs-target-G_3SG nanocomplex in the presence of 20 nM β-thalassemia gene fragment target, (B) blank NFs, and (C) NFs-target-G_3SG in the absence of target.

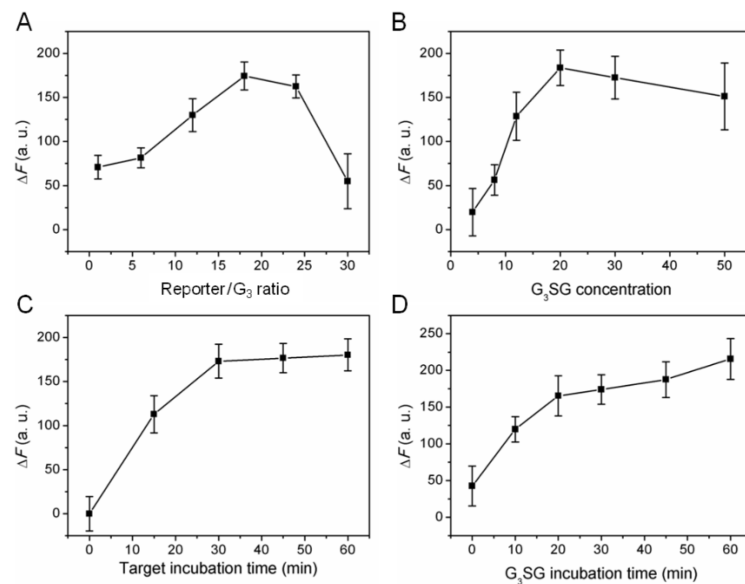

Fig. S6 Fluorescence increment (Δ*F*) of the proposed sensors to 1 nM β-thalassemia gene fragment target at different (A) reporter/G_3 ratio, (B) G_3SG concentration,(C) target incubation time, and (D) G_3SG incubation time. The optimized sensing conditions were chosen as follow: the number of reporter/G_3 ratio was 16, the G_3SG concentration was 20 nM, the target incubation time was 30 min, and the G_3SG incubation time was 30 min.

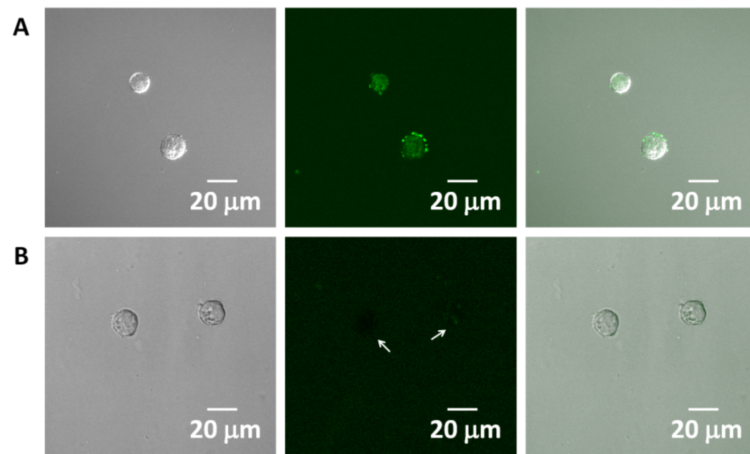

Fig. S7 Confocal fluorescence microscopy images showing the HeLa cells (A) and HEK293T cells (B) incubated with G_3SG-sgc8. The cell density was 1×10^5 cells/mL, and the concentration of G_3SG-sgc8 was 10 nM. As could be seen, the G_3SG-Sgc8 specifically bound to the surfaces of HeLa cells.

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