Electronic Supplementary Information

Assembly of DNA-functionalized gold nanoparticles on electrospun nanofibers as a fluorescent sensor for nucleic acids

Huaming Wang, Dingzhong Wang, Zhou Peng, Wei Tang, Na Li and Feng Liu*

Beijing National Laboratory for Molecular Science, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.

*To whom correspondence should be addressed. Fax: 86-10-62751708;

Tel: 86-10-62761187; E-mail: liufeng@pku.edu.cn

Materials

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were purchased from Beijing Chemical Works (Beijing, China). Cellulose acetate (CA) (M_w=150 kDa), n-dodecanethiol (DT), and 6-mercapto hexanol (MCH) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). N, N-Dimethylacetamide (DMAC) was purchased from Beijing Yili Fine Chemical Company (Beijing, China). Tris(hydroxymethyl) aminomethane (Tris) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade, and deionized water was used in all experiments.

All HPLC-purified DNA oligonucleotides were ordered from Sangon Inc. (Shanghai, China), and their sequences are listed in Table S1. The DNA oligonucleotides were dissolved in Tris buffer (100 mM Tris, 100 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH=8.3) with a final concentration of 10 μM and stored in the dark at 4 °C.

Table S1 DNA oligonucleotide sequences mainly used in this study.

Name	Sequences（5' to 3'）
Capture probe	$HS(CH_2)_6$-TTTTTGCTTTGTTCTGGATTTCGCAGGT
Reporter probe	GAAATCCAGAACAAAGCA-FAM
BRCA I gene fragment	ACCTGCGAAATCCAGAACAAAGCA
T-SM sequence	ACCTGTGAAATCCAGAACAAAGCA
A-SM sequence	ACCTGAGAAATCCAGAACAAAGCA
G-SM sequence	ACCTGGGAAATCCAGAACAAAGCA
Random sequence	TTGCCTACGCCACCAGCTCCAACT

The toehold domain in the capture probe is underlined. The bases at the mutational position are highlighted in red.

Characterization

The CA electrospun nanofibrous membranes 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. The morphology characterization of the DNA-AuNP-CANF nanocomposite membranes were performed using an S-4800 scanning electron microscope (SEM) (Hitachi, Tokyo, Japan) and a Tecnai F20 transmission electron microscopy (TEM) (FEI, USA). Fluorescence spectra were determined using an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). UV-vis absorption was recorded on a U-3010 spectrophotometer (Hitachi, Tokyo, Japan).

Fabrication of CA electrospun nanofibrous membrane

0.18 g CA and various amount of thiol compound were co-dissolved into 1.5 mL DMAC/acetone (1:2, v/v) mixed solvent and stirred to obtain homogeneous solution. The solution was loaded into the electrospinning setup to fabricate the CA electrospun nanofibers (CANFs). The typical electrospinning parameters were as follow: the voltage was 20.0 kV, the collecting distance was 15 cm, the solution feeding rate was

0.40 mL/h, and the electrospinning time was 30 min. Hydrophobic glass slides mounted on the rotary collector were used to collect the CANF membranes. The membranes were then dried in the vacuum oven at 40 °C overnight to remove the residual solvent.

Synthesis and DNA functionalization of gold nanoparticles

Gold nanoparticles (AuNPs) stabilized with citrate were synthesis according our pervious report.[1] The TEM image showed the size distribution of the obtained AuNPs was 10–15 nm. The concentration of the prepared AuNPs was estimated to be 13 nM using the absorbance at 520 nm, with the appropriated extinction coefficient.[2]

For the DNA functionalization of the AuNPs, 10 μM reporter probe and 10 μM capture probe were first hybridized in Tris buffer solution at the molar ratio of 1.05:1, and then added to the AuNPs solution at the molar ratio of 92:1. After incubated at room temperature for 12 h, the obtained DNA-functionalized gold nanoparticles (DNA-AuNPs) solution was used for the decoration on CANF membrane without the need of centrifugal separation.

Fabrication of DNA-AuNP-CANF sensing membrane

The CANF membrane was successively rinsed with deionized water and Tris buffer. 400 μL of 10 nM DNA-AuNPs solution containing 0.10 M NaCl was dripped onto the CANF membrane. The mixture was incubated at room temperature for 8 h, allowing assembly of the DNA-AuNPs on the CANF membrane through gold-sulfur bond. The obtained DNA-AuNP-CANF nanocomposite membrane was rinsed thoroughly with Tris buffer until no fluorescence was observed in the eluate.

For comparison, the DNA-AuNP-CA dense film and the DNA-gold film were prepared through the following procedures. The CA dense film was fabricated by spin-casting the CA/DT solution using a KW-4A Spin Coater (Beijing Casue Technology Co., Ltd., Beijing, China) at 1200 rpm for 10 min. Then DNA-AuNPs was assembled on the CA dense film to fabricate the DNA-AuNP-CA dense film

under the same condition as the DNA-AuNP-CANF membrane. The DNA-gold film was prepared by directly incubating commercial gold film (Hrbio Co. Ltd., Beijing, China) with 400 μL of 500 nM DNA probes for 8 h. The SEM images (Fig. S10) show the morphology of the DNA-AuNP-CA dense film and the DNA-gold film.

DNA sensing procedure

For the DNA sensing via the toehold-mediated strand displacement reaction, the glass slide covered with the DNA-AuNP-CANF sensing membrane was placed horizontally in a weighing bottle (60 mm × 30 mm). 400 μL of the target DNA in Tris buffer solution (containing 100 mM Tris, 100 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH = 8.3) was dropped on the sensing membrane, and the bottle was sealed with a ground glass stopper to prevent evaporation. After incubation for 1 h, the glass slide covered with sensing membrane was taken off from the solution, and then the fluorescence spectrum of the solution was recorded from 505 to 600 nm with the excitation wavelength of 494 nm in a micro fluorometric cell (10 mm × 3 mm).

Quantification of the DNA probe density on the sensing membrane

The number of DNA probes loaded on the DNA-AuNP-CANF sensing membrane was determined according to the literature procedure with some modification.[3] 400 μL Tris buffer solution containing 10 mM 6-mercapto hexanol (MCH) was added to a DNA-AuNP-CANF membrane of 1.4×2.5 cm^2 to chemically displace the DNA probes. After incubation at room temperature overnight with slight shaking, the membrane was taken out and rinsed with Tris buffer. The total supernatant containing the free DNA probes was diluted to 5 mL and its fluorescence intensity was measured at 520 nm. A standard curve of the DNA probe from 0.5 to 50 nM was also prepared under the same buffer, pH and salt concentration. The DNA probe density (d(DNA), pmol cm^{-2}) was calculated via dividing the amount of MCH exchanged DNA probes from the sensing film (n(DNA), pmol) by the area of the slide covered with the sensing film (S(film), cm^{-2}):

$$d(\mathrm{DNA}) = \frac{n(\mathrm{DNA})}{S(\mathrm{film})} \quad (1)$$

From the equation (1), the DNA densities on the DNA-AuNP-CANF membrane, the DNA-AuNP-CA dense film, and the DNA-gold film were calculated to be 71, 7.1, and 1.2 pmol cm^{-2}, respectively.

The DNA probe densities on the DNA-AuNP-CA dense film and the DNA-gold film were calculated using the same approach.

We measured the absorbance of the AuNP solution before and after incubation with the CANF membrane to estimate the amount of AuNPs immobilized on the DNA-AuNP-CANF membrane. And the number of DNA probes on each AuNP was calculated via dividing the amount of DNA probes by the amount of AuNPs on the DNA-AuNP-CANF membrane. It could be seen from Fig. S11b that the fluorescence response of the sensing membrane initially increases with the number of DNA probes on each AuNP, but decreases dramatically after the number grows larger than 82. The result may be attributed to that the crowded DNA probes prevent the target from approaching the toehold binding sites on the surface efficiently. Therefore, the optimized experiment condition was chosen as the number of DNA probes on each AuNP was 82.

Effect of size and concentration of AuNPs on the fabrication and the sensitivity of the DNA-AuNP-CANF membranes

Gold nanoparticles of different diameters were synthesized according to the reference.[4] The average diameters calculated from 50 nanoparticles in the TEM images are 4.6±0.9 nm, 12.7±1.6 nm, 19.9±2.9 nm, and 23.7 ± 3.2 nm, respectively (Fig. S1). And the SEM and TEM images of DNA-AuNP-CANF membranes with different AuNP diameters are shown in Fig. S2 and Fig. S3. As could be seen, the AuNPs with diameters of 12.7 nm and 19.9 nm are distributed on the nanofiber surface with a high density and only slight aggregation. The 4.6 nm AuNPs tend to aggregate seriously on the nanofiber surface, while the 23.7 nm AuNPs are relatively inactive to assemble on the nanofibers and generate nanocomposite with low AuNP

density.

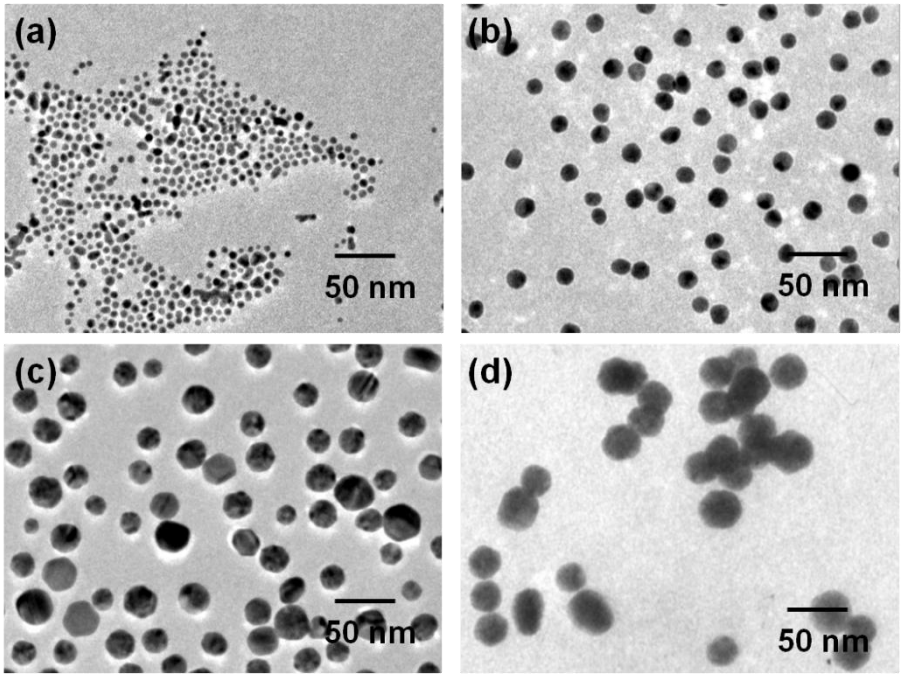

Fig. S1 TEM images of the AuNPs with average diameters of 4.6±0.9 nm (a), 12.7±1.6 nm (b), 19.9±2.9 nm (c), and 23.7 ± 3.2 nm (d).

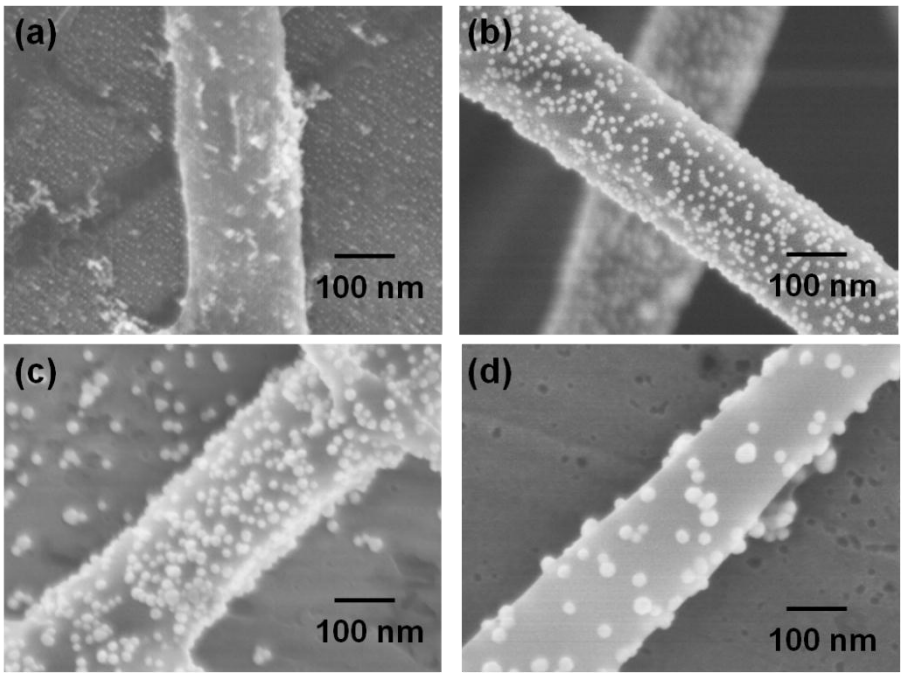

Fig. S2 SEM images of the DNA-AuNP-CANF fabricated with AuNPs of different diameters: 4.6 nm (a), 12.7 nm (b), 19.9 nm (c), and 23.7 nm (d).

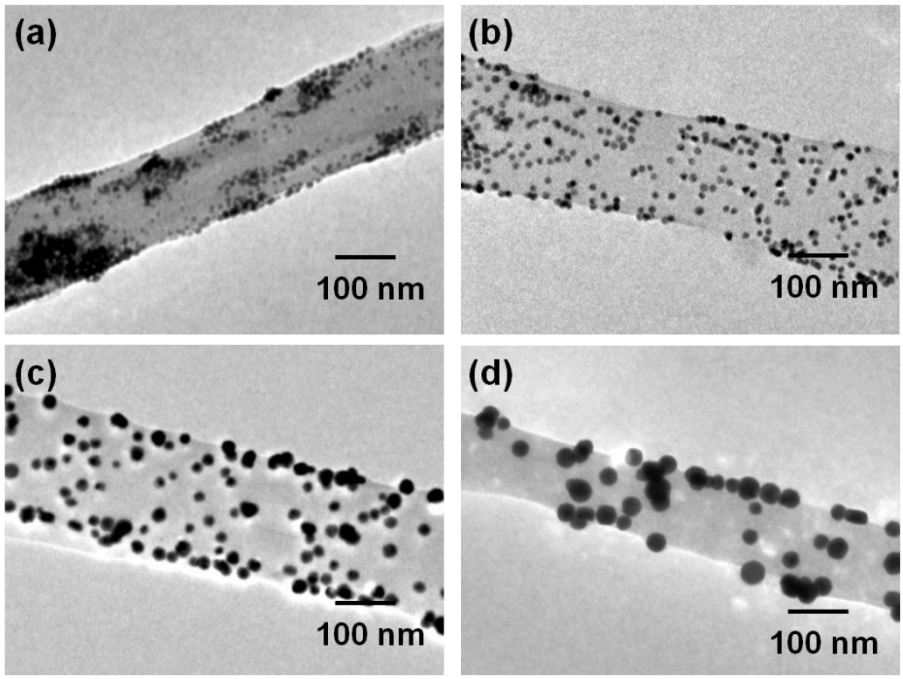

Fig. S3 TEM images of the DNA-AuNP-CANF fabricated with AuNPs of different diameters: 4.6 nm (a), 12.7 nm (b), 19.9 nm (c), and 23.7 nm (d).

We also investigated the effect of size and concentration of the DNA-AuNPs on the sensing sensitivity of the DNA-AuNP-CANF membrane. As shown in Fig. S4a, the nanocomposite membrane with 12.7 nm AuNPs exhibits the highest sensitivity for BRCA I gene fragment. The result can be explained by that the smaller AuNPs tends to assemble on the nanofibers with higher density, but the aggregation of 4.6 nm AuNPs reduced the specific surface area of the nanocomposite.

Moreover, the sensitivity of the DNA-AuNP-CANF sensor is positively correlated to the AuNPs concentration during fabrication (Fig. S4b). Here 10 nM is the highest AuNP concentration obtained after the DNA functionalization of undiluted AuNP solution. Therefore, we chose 12.7 nm AuNPs at 10 nM for fabricating the DNA-AuNP-CANF nanocomposite membrane.

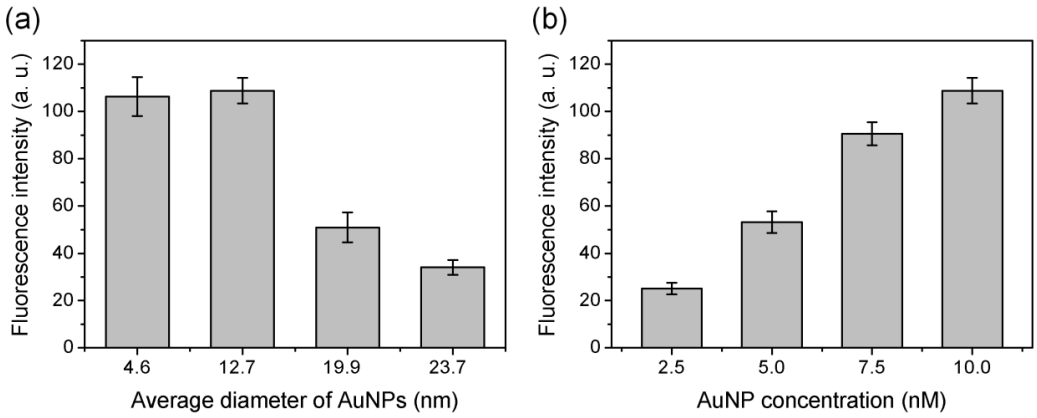

Fig. S4 Fluoresence intensities of the DNA-AuNP-CANF membranes fabricated with AuNPs of different sizes (a) and 12.7 nm AuNPs at different concentrations (b) for 10 nM BRCA I gene fragment sensing. The error bars represent the standard deviation of three measurements.

Effect of the species and concentration of thiol compounds on the fabrication and the sensitivity of the DNA-AuNP-CANF membranes

6-Mercapto hexanol (MCH) and n-dodecanethiol (DT) were chosen as the thiol compounds to fabricate the mercapto functionalized CANFs. The morphology of the nanofibers containing MCH after incubation with DNA-AuNPs is shown in Fig. S5. As can be seen, only a few nanoparticles have assembled on the nanofiber surface, while large numbers of aggregated gold nanoparticles are observed. The possible explanation for the phenomenon is that the water-soluble MCH in the nanofibers leaked into the solution and promoted the aggregation of the gold nanoparticles.

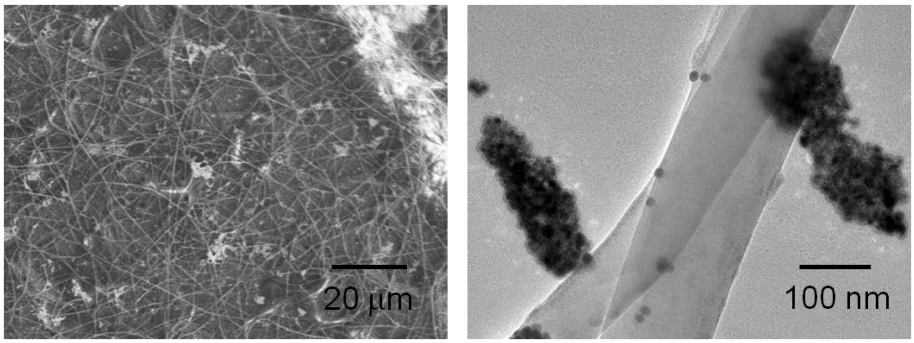

Fig. S5 SEM (a) and TEM (b) images of the nanocomposite membranes fabricated using MCH as the thiol reagent.

To prevent this, we used another thiol compound, n-dodecanethiol (DT) which contains a longer alkyl chain to increase its hydrophobicity. We varied the concentration of DT doped in the CANFs. As shown in Fig. S6, higher AuNP density on the surface of the DNA-AuNP-CANF nanocomposite can be observed with the increasing percentage content of DT from 15% to 50%. But when the DT percentage reaches 66%, the morphology of the nanofibers becomes irregular with lots of beads in the structure, which is expected to lower the surface area-to-volume ratio of the nanocomposite. Study on the fluorescence responses for 10 nM BRCA I gene fragment (shown in Fig. S7) also indicate that the sensor exhibits highest sensitivity when the percentage content of DT is 50%. Therefore, 180 μL DT (50% DT percentage content) was chosen as the optimized condition for fabricating the DNA-AuNP-CANF nanocomposite.

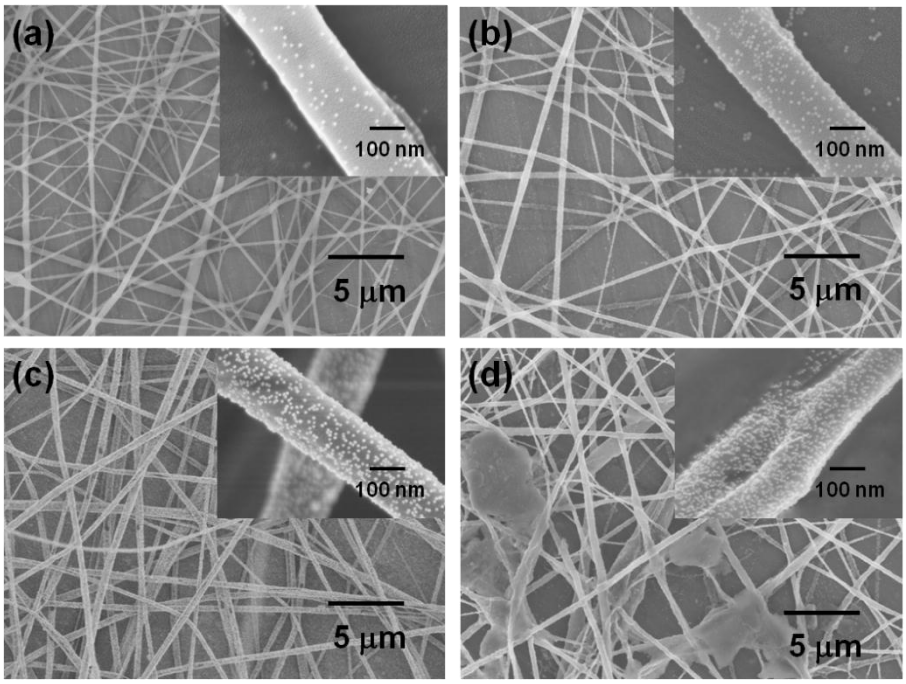

Fig. S6 SEM images of the DNA-AuNP-CANF membrane fabricated using DT percentage content of 15% (a), 33% (b), 50% (c), and 66% (d). The insert shows the further magnified SEM images of the nanocomposite.

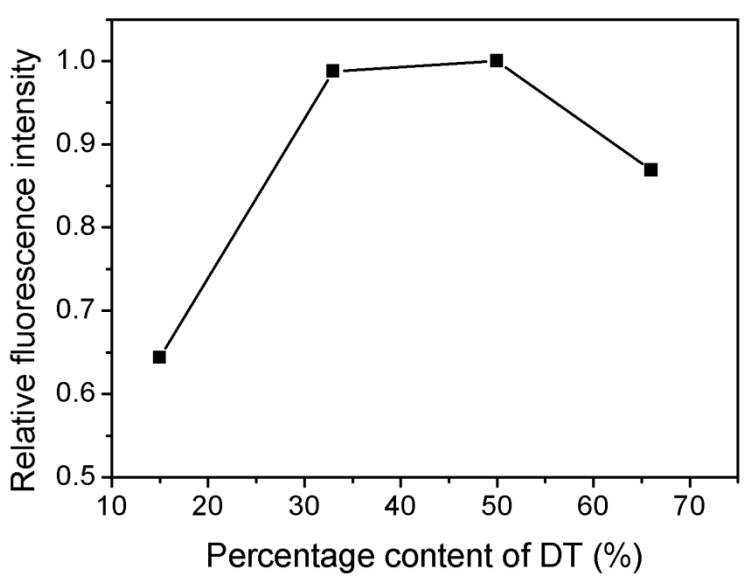

Fig. S7 Relative fluorescence intensity of the DNA-AuNP-CANF sensor to 10 nM BRCA I gene fragment when using different DT percentage content for nanocomposite fabrication.

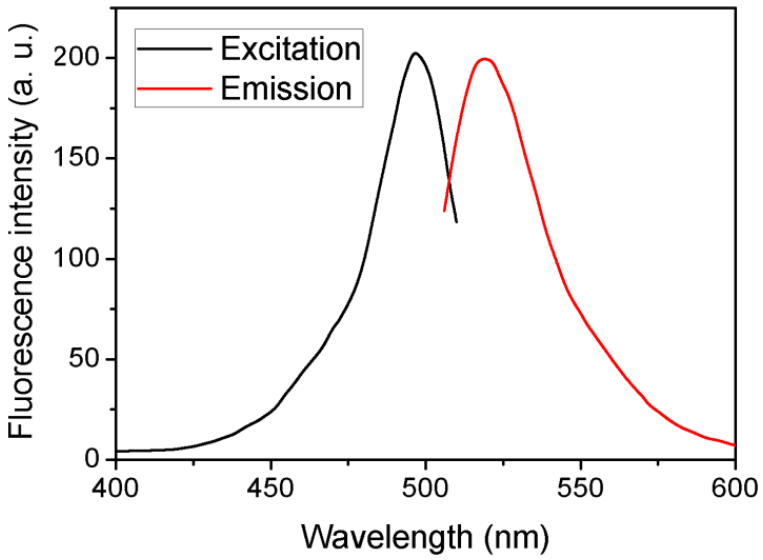

Fig. S8 Excitation and emission spectra of 10 nM 5-carboxyfluorescein (FAM) labelled reporter probe in Tris buffer. The maximum excitation and emission wavelengths are at 494 and 520 nm, respectively.

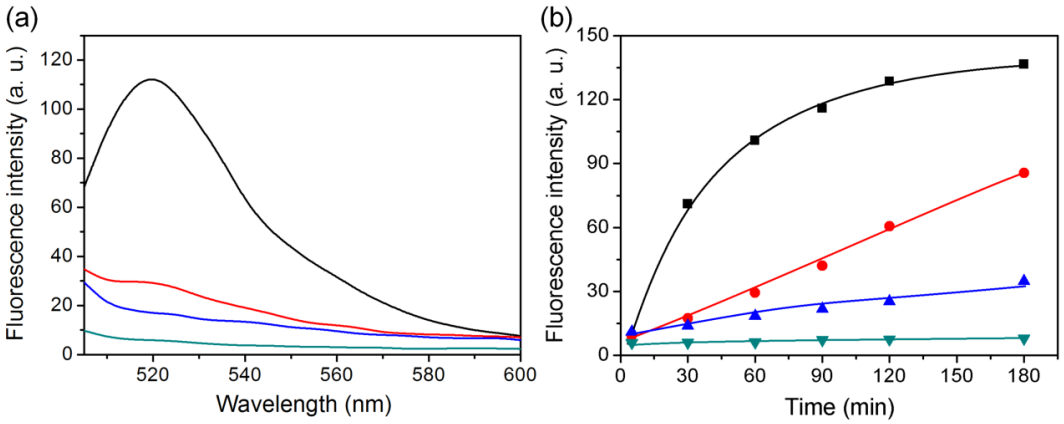

Fig. S9 Fluorescence spectral (a) and time-dependent fluorescence intensity (b) of the DNA-AuNP-CANF sensor to a series of targets from top to bottom: BRCA I gene fragment, A-SM sequences, random sequences, and Tris buffer blank. The concentration of each sequence is 10 nM. 1 h was chosen as the optimized sensing time in order to obtain better discrimination capacity.

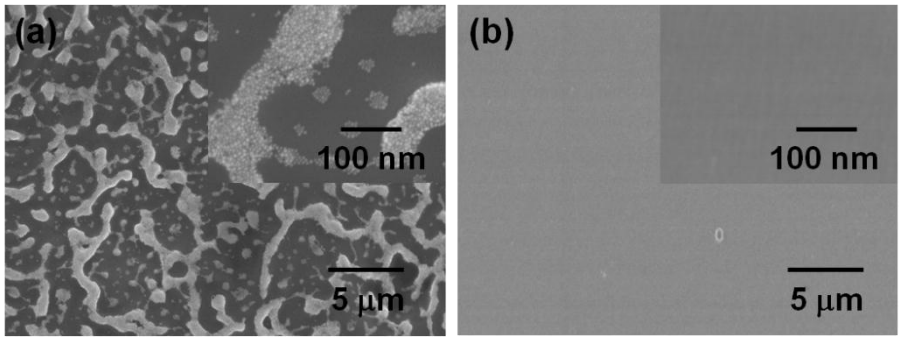

Fig. S10 SEM images for the DNA-AuNP-CA dense film (a) and the DNA-gold film (b).

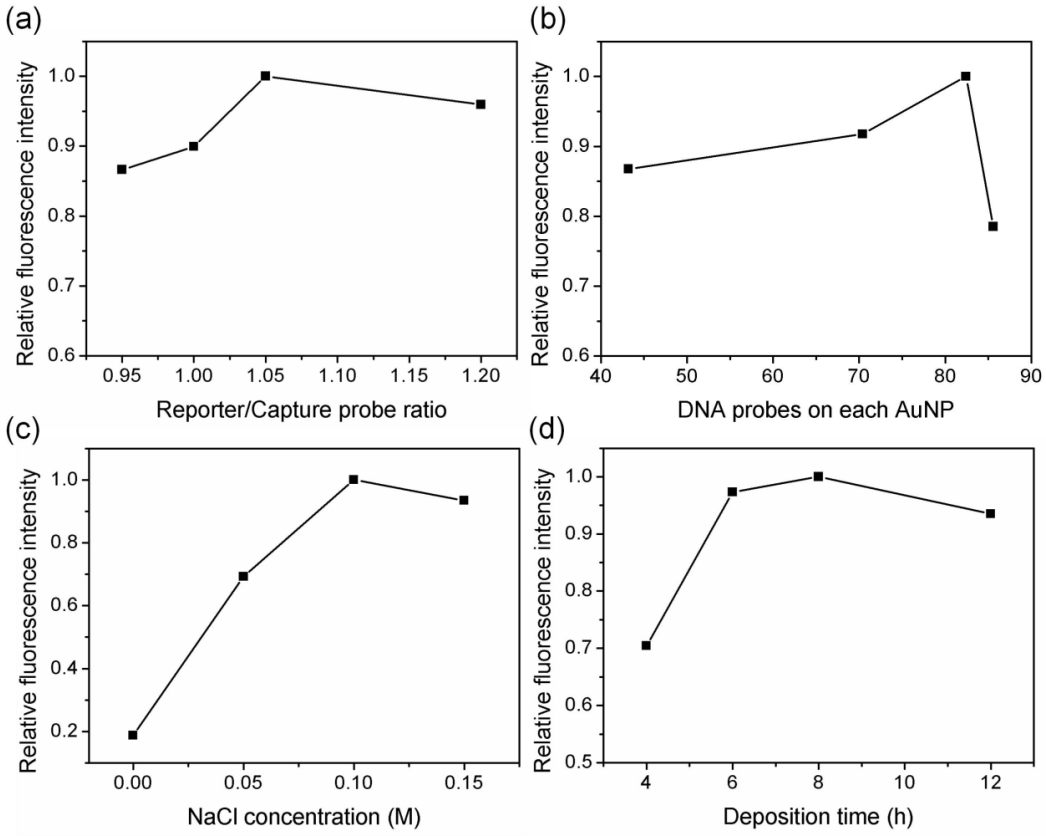

Fig. S11 Relative fluorescence intensity of the DNA-AuNP-CANF sensor to BRCA I gene fragment at different report probe/capture probe ratio (a), number of DNA probes on each AuNP (b), NaCl concentration (c), and deposition time (d). The optimized conditions for the sensor fabrication were chosen as the reporter/capture probe ratio was 1.05:1, the number of DNA probes on each AuNP was 82, the NaCl concentration was 0.10 M, and the deposition time was 8 h.

Kinetic analysis of the toehold-mediated strand displacement on the sensing membranes

The kinetics of the toehold-mediated strand displacement reaction (SDR) was reported using a biomolecular reaction model.[5] For our system, the initial labeled duplex (CR*) interacts with the target (T) to generate a secondary duplex (CT), releasing the fluorescent labeled reporter probe (R*). The rate of the reaction expresses in terms of the production of the species R* as equation (2)

$$CR^* + T \xrightarrow{k} CT + R^*$$

$$\frac{\mathrm{d}[\mathrm{R}^*]}{\mathrm{d}t} = k[\mathrm{CR}^*][\mathrm{T}] = k[\mathrm{CR}^*]([\mathrm{T}]_0 - [\mathrm{R}^*]) \quad (2)$$

where [CR*], [T], and [R*] are the concentrations of the DNA duplex probe, the target DNA, and the fluorescent labeled reporter probe, respectively. $[\mathrm{T}]_0 = [\mathrm{T}] + [\mathrm{R}^*]$ denotes the initial concentration of the DNA target. *t* is the time of the displacement reaction. *k* is the rate constant of the SDR which depends on the DNA sequences as well as the ambient temperature and salt concentrations.

In our case, the DNA probes immobilized on the surface of the sensing membrane are abundant comparing to the target DNA, we define the apparent displacement rate $k_{app} = k\,[\mathrm{CR}^*]$ as a constant to obtain equation (3):

$$\frac{\mathrm{d}[\mathrm{R}^*]}{\mathrm{d}t} = k_{\mathrm{app}}([\mathrm{T}]_0 - [\mathrm{R}^*]) \quad (3)$$

The solution of this differential equation is shown below:

$$\ln\frac{([\mathrm{T}]_0 - [\mathrm{R}^*])}{([\mathrm{T}]_0 - [\mathrm{R}^*]_0)} = -k_{\mathrm{app}}t \quad (4)$$

$[\mathrm{R}^*]_0$ is the concentration of reporter probe at $t = 0$, which is zero for our system. The fluorescence intensity (*F*) of the system is proportional to the concentration of the fluorescent labeled reporter probe [R*]:

$$F \propto [\mathrm{R}^*] = [\mathrm{T}]_0 - ([\mathrm{T}]_0 - [\mathrm{R}^*]_0)\exp\left(-k_{\mathrm{app}}t\right) \quad (5)$$

We simulated the time-dependent fluorescence intensity of the three sensing membranes for the 10.0 nM BRCA I gene fragment using the equation (5), and the results are shown in Table S2 in comparison with the DNA probe densities on the three sensing membranes.

Table S2 Apparent displacement rate constants of the three sensing membranes to 10 nM BRCA I gene fragment.

Sensing membranes	DNA-AuNP-CANF membrane	DNA-AuNP-CA dense film	DNA-gold film
k_{app} **(min^{-1})**	1.82×10^{-2}	1.34×10^{-3}	3.59×10^{-4}
DNA probe density (pmol cm^{-2})	71	7.1	1.2

The apparent rate constant $k_{app} = k$ [CR*] of the DNA-AuNP-CANF membrane is 13.5 and 50.7 times respectively to the DNA-AuNP-CA film and the DNA-gold film. Considering the sequences of the DNA probes and the experimental conditionals are the same for the three sensing membranes, the rate constant k of the SDR should be equal in value. Therefore, the kinetic responses of the sensing membranes are controlled by [CR*], which depends on the amount of the immobilized DNA probes. The result proves that the high sensitivity of the DNA-AuNP-CANF membrane is attributed to the high DNA probe density on the membrane.

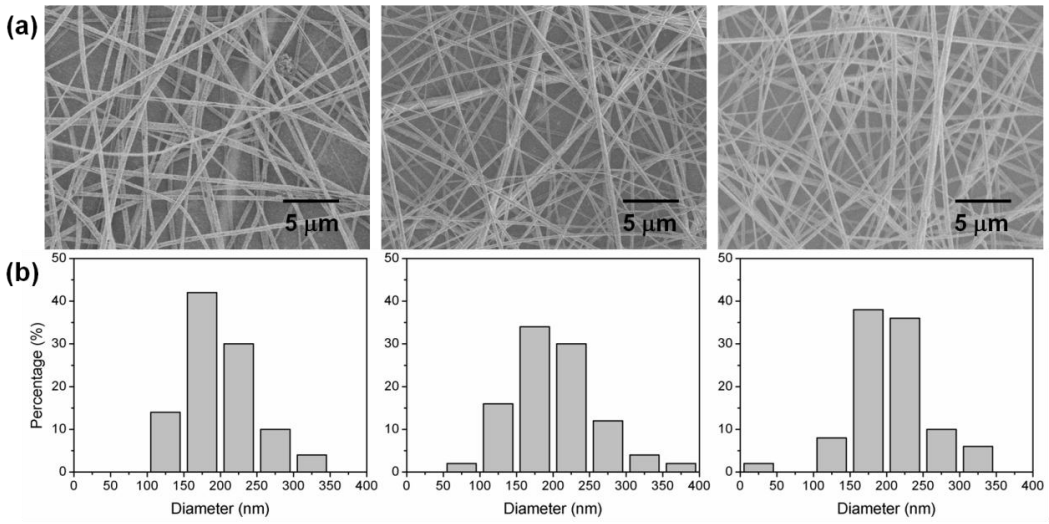

Fig. S12 SEM images (a) and size disperse histograms (b) of the DNA-AuNP-CANF membranes produced from three different batches.

References

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