Ultrasensitive Detection of Nucleic Acids Based on Dually Enhanced Fluorescence Polarization

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Theoretical analysis of NSET-enhanced fluorescence polarization

The relationship between the concentration and fluorescence polarization of fluorophoresis given by[1, 2]

$$\frac{1}{P} = \frac{1}{P_0} + Ac\tau \tag{S.1}$$

where$P$ is fluorescence polarization of fluorophores at the concentration of c, $P_0$is fluorescence polarization of infinite dilution of fluorophores. A can be seen as a constant at low concentration and τ is the excitation state lifetime of fluorophores. So fluorescence polarization of fluorophores can be given by

$$P = \frac{P_0}{1 + Ac\tau} \tag{S.2}$$

When energy transfer occurs, the lifetime is shortened and can be given by

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \tag{S.3}$$

where$E$ is the energy transfer efficiency, $\tau_{DA}$and $\tau_D$are the lifetime of fluorophores with and without quenchers, respectively. So the fluorescence polarization of fluorophores conjugated with quenchers is given by

$$P = \frac{P_0}{1 + A_{DA}c(1-E)\tau_D} \tag{S.4}$$

Thus it can be considered that the effective concentration becomes (1-E)c, which improves the fluorescence polarization value.

Besides, the increase of the molecular volume of fluorophores due to quencher conjugation can also raise the polarization value. The constant A is inversely proportional to the rotational relaxation time (r) according to formula (S.1) and the dependence of polarization on the rotational relaxation time[3]

$$\frac{1}{P}-\frac{1}{3}=\left(\frac{1}{P_0}-\frac{1}{3}\right)\left(1+\frac{3\tau}{\Gamma}\right) \tag{S.5}$$

When fluorophores are conjugated with quenchers, the rotational relaxation time is prolonged and the constant *A* decreases, which enhances the fluorescence polarization in another respect.

Characterization of AuNPs

We obtained the SEM image and analyzed the size of AuNPs using ImagJ software. LSP absorbance spectrum of AuNPs was compared with the emission spectrum of Alexa488 dye.

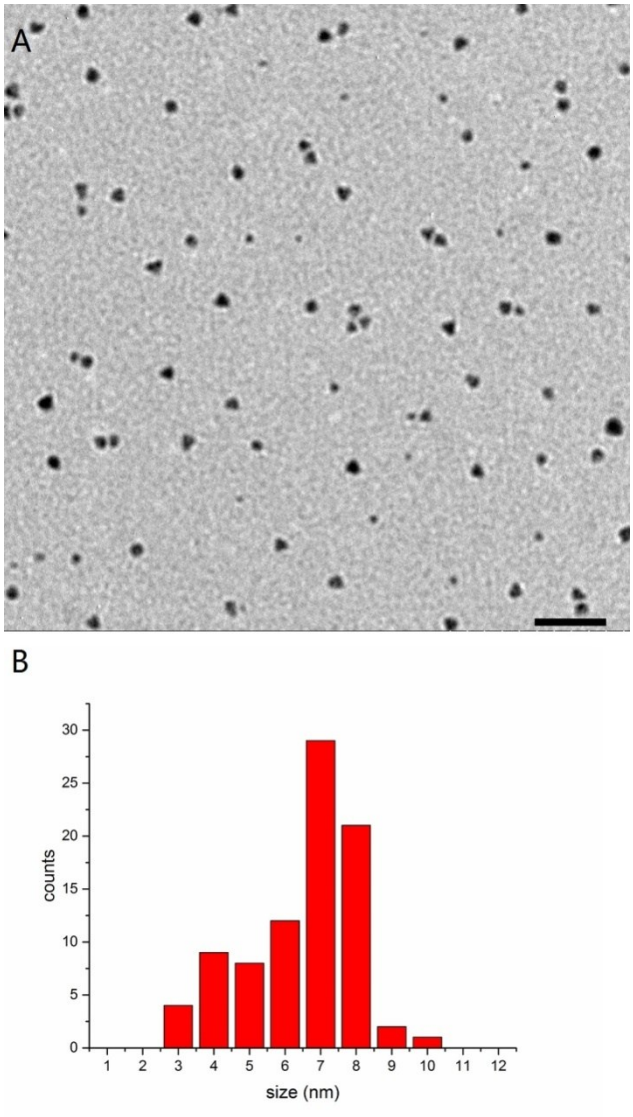

Fig.S1.TEM image of AuNPs. (A) TEM image of AuNPs obtained by Hitachi H-7650 at the ×50k magnification and the 80kV accelerating voltage. (B) Size distribution of AuNPs. The average diameter was calculated to be 6.55nm.

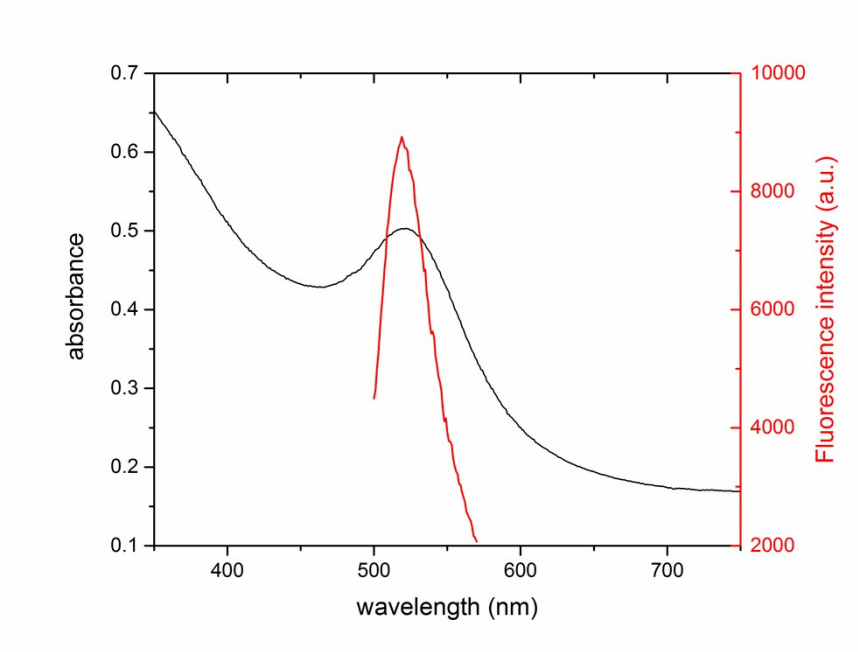

Fig. S2. LSP absorbance spectrum of AuNPs (black curve with the left-Y axe) and the emission spectrum of the Alexa488 dye (red curve with the right-Y axe). There exists perfect overlap of LSP absorbance band of AuNPs with the emission band of Alexa488.

Molar ratio of Alexa488 and AuNPs

10nM Alexa488 dye was mixed with dsDNA-AuNPs of different final concentrations and fluorescence intensity and polarization of Alexa488 were detected after 30 minutes in order to determine the quantitative relation of Alexa488 and AuNPs during synthesis.

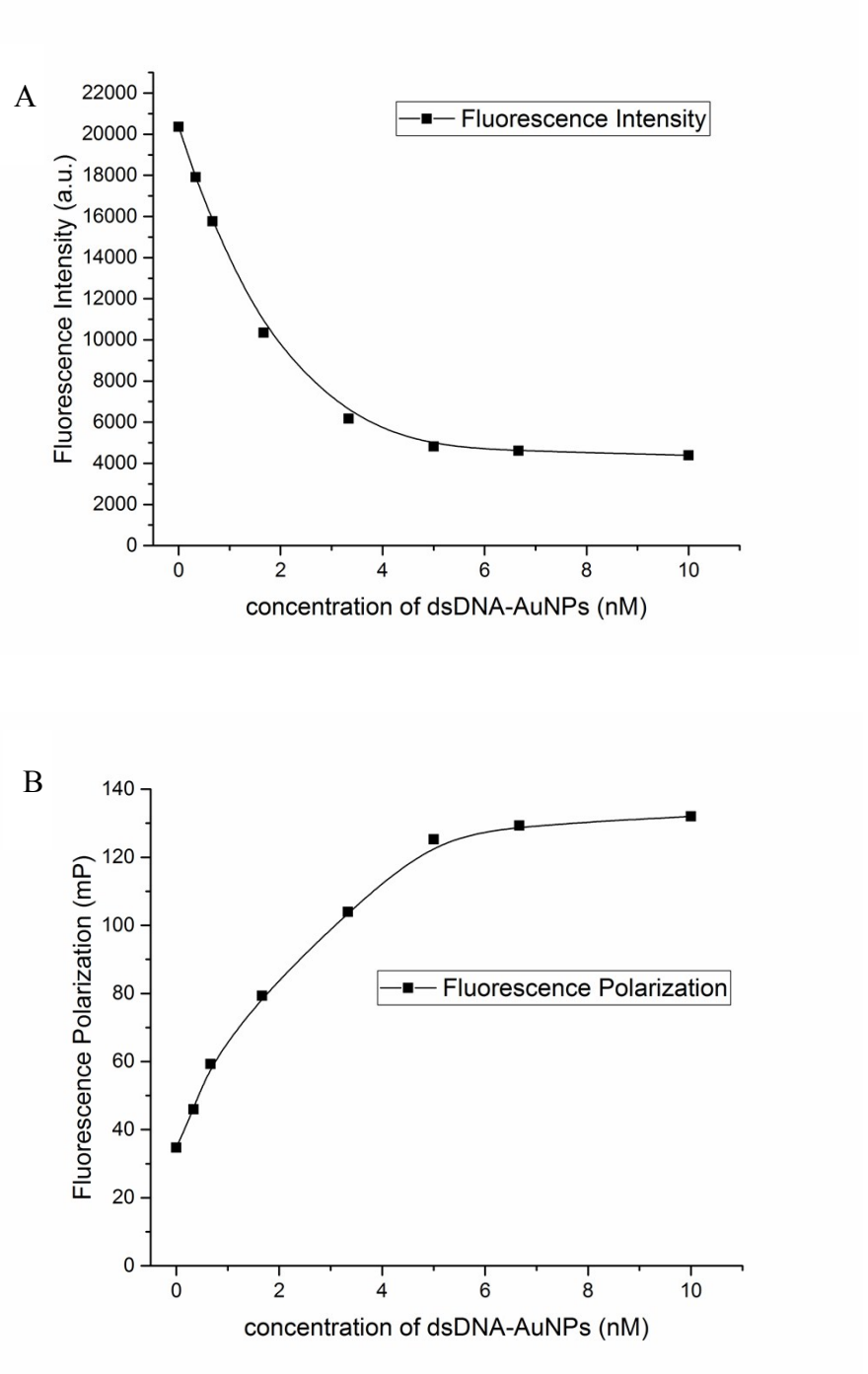

Fig. S3. Optical quenching of Alexa488 with dsDNA-AuNPs conjugates of different concentrations. (A) Fluorescence intensity. (B) Fluorescence polarization.

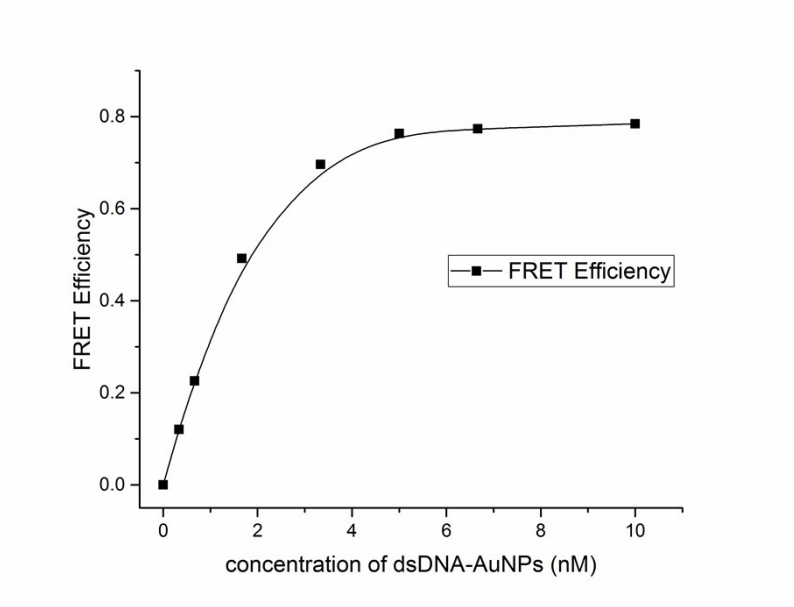

Fig. S4. Quenching efficiency changed with varied molar ratio of Alexa488 and AuNPs.

DNA detection in DMEM solution

To validate the feasibility of this method in real biological samples, the competitive displacement assay for DNA detection was conducted in DMEM solution with 150mM of sodium ions.

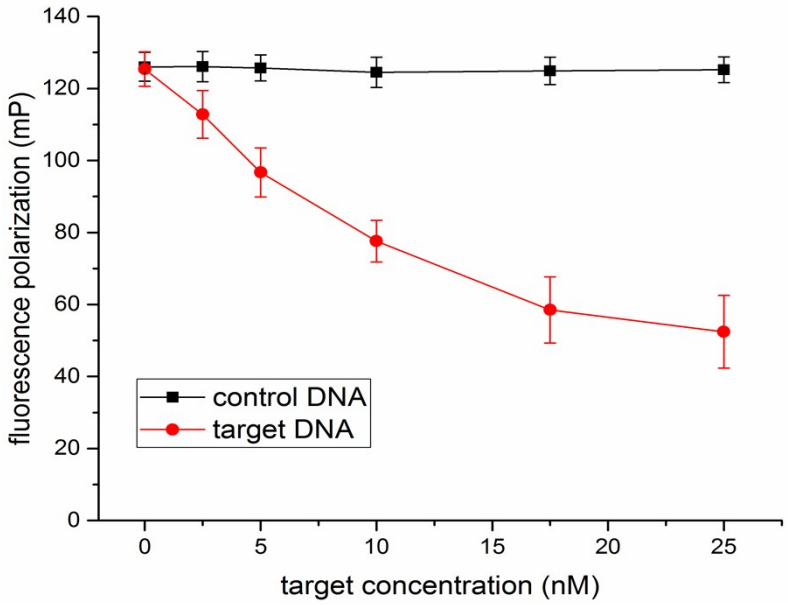

Fig. S5. Fluorescence polarization based DNA detection in DMEM solution. Error bars were obtained from three independent experiments.

Table S1. Analytical performance between the reported methods and the proposed method.

Technique	Target molecule	Detection limit	Ref
FRET	DNA	12nM	[4]
Oligonucleotide replacement	DNA	nM level	[5]
Electrochemiluminescence	miRNA	1.1nM	[6]
Surface plasmon resonance	miRNA	1nM	[7]
Quartz crystal microbalance	miRNA	400pM	[8]
Fluorescence polarization	DNA	500pM	[9]
Fluorometric	DNA	20pM	[10]
Fluorescence polarization	DNA	372pM	this work

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