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## 1. Experimental Section

### 1.1. Characterization of the Mass Amplifying Probe

The silicon wafers were immersed in deionized water for a long time and then rinsed with ethanol solution. A total of 10  $\mu\text{L}$  of the mass amplifying probe (1 $\times$ ) were deposited respectively on the cleaned silicon wafers for 1 h, then the surface of silicon wafers was gently rinsed with deionized water and dried in a vacuum drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd. Shanghai, China) overnight. The morphologies of the mass amplifying probe were observed and recorded using an S-4800 scanning electron microscope (Hitachi, Japan).

### 1.2. Agarose Gel Electrophoresis

The reaction products were identified by 1.5% of agarose gel electrophoresis for 45 min (gel prepared in 1 $\times$ TAE buffer supplemented with 10 mM  $\text{MgCl}_2$  and 0.005% (v/v) ethidium bromide solution). The gels were visualized under UV light using a BGdsAUTO520 portable UV apparatus (Baygene, Beijing, China).

### 1.3. miRNA-21 Detection in Serum Samples

Human serum samples were acquired from Southwest University Hospital. Abiding by the ethical standards of the institutional committee of Southwest University, we got informed consents from the volunteer of this work. MiRNA-21 of different concentrations were dissolved in human serum as real sample. Then, the above serum solution was analyzed by the proposed method.

## 2. FA Measurements

Fluorescence measurements were carried out on the F-2500 fluorescence spectrophotometer with an excitation wavelength of 560 nm and emission was detected at 584 nm. The slits for both excitation and emission of 5 nm. The fluorescence anisotropy ( $r$ ) of the test solution was calculated by

$$r = \frac{I_{VV} - G \times I_{VH}}{I_{VV} + 2G \times I_{VH}} \quad (1)$$

and

$$G = \frac{I_{HV}}{I_{HH}} \quad (2)$$

where  $I$  corresponds to the fluorescence intensity and the subscripts define the orientation  $H$  and  $V$  for horizontal and vertical of the excitation and emission polarizers, respectively.  $G$  is the grating factor of the fluorescence spectrophotometer, which is used to correct the wavelength response to polarization of the emission optics and detectors.

FA signal was measured on an F-2500 fluorescence spectrophotometer, which was equipped with a polarization filter (Hitachi, Tokyo, Japan).

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## Oligonucleotide Sequences

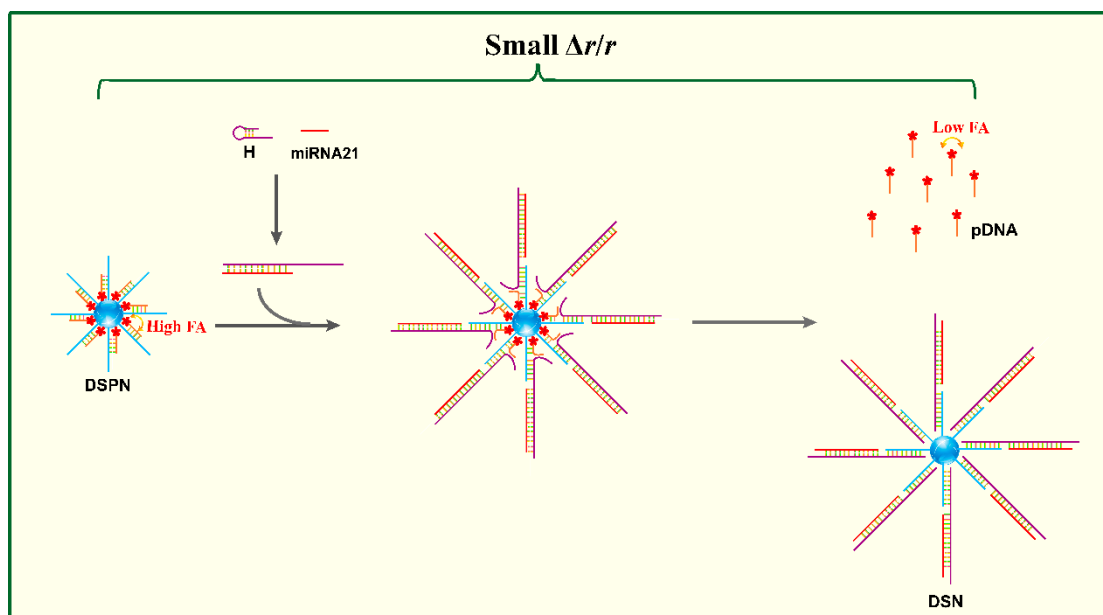
**Table S1.** Oligonucleotides Sequence Used in this Study.

Strand	Sequence (from 5' to 3')
T1	GTGTGCCTATTATGCTCCTCCTGTGTGCCTATTATGTCTCCTCCTC AGCTTCATCAACTAGTTCGTA
T2	AGGAGGAGACATAATAGGCACACTGACGAACTAGTTGATGAAG CTG
P1	AACTAGTTGATGAAGCTGGACATAATAGGCACACGACATAATA GGCACAC
P2	CAGCTTCATCAACTAGGTGTGCCTATTATGTCTC
As-A	GTGCCTATTATGTCGTGTGCCTATTATGTCCAGCTT
As-B	GCACACCTAGTTGATGAAGC
Initiator	TGACGAACTAGTTGATGAAGCTG
Linker	Biotin-TTT TT ACAGCTTCATCAACTAGTTCGTCA
pDNA	GTTGATGAAGCTGT-TAMRA
H1	CAGACTGATGTTGATGACGAACTAGTTGATGAAGCTGT TCAACATCAGTCTGATAAGCTA
H2	TGACGAACTAGTTGATGAAGCTGTCAGACTGATGTTGAACAGCT TCATCAACTAGTTCGTCATCAACATCAG
H1-1	TTATCAGACTGATGTTGATGACGAACTAGTTGATGAAGCTGT TCAACATCAGTCTGATAAGCTA
H2-1	TGACGAACTAGTTGATGAAGCTGTTTATCAGACTGATGTTGAAC AGCTTCATCAACTAGTTCGTCATCAACATCAGTCTG
H1-2	ATCAGACTGATGTTGATGACGAACTAGTTGATGAAGCTGT TCAACATCAGTCTGATAAGCTA
H2-2	TGACGAACTAGTTGATGAAGCTGTATCAGACTGATGTTGAACAG CTTCATCAACTAGTTCGTCATCAACATCAGTC
H1-3	GACTGATGTTGATGACGAACTAGTTGATGAAGCTGT TCAACATCAGTCTGATAAGCTA
H2-3	TGACGAACTAGTTGATGAAGCTGTGACTGATGTTGAACAGCTTC ATCAACTAGTTCGTCATCAACATC
H1-4	CTGATGTTGATGACGAACTAGTTGATGAAGCTGT TCAACATCAGTCTGATAAGCTA
H2-4	TGACGAACTAGTTGATGAAGCTGT CTGATGTTGAACAGCTTCATCAACTAGTTCGTCATCAACA
Linker1	Biotin-ACAGCTTCATCAACTAGTTCGTCA
Linker2	Biotin-TTT TT TTTT ACAGCTTCATCAACTAGTTCGTCA
Linker3	Biotin-TTT TT TTTT TTTT ACAGCTTCATCAACTAGTTCGTCA
Linker4	Biotin-TTT TT TTTT TTTT TTTT ACAGCTTCATCAACTAGTTCGTCA
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
Mis-1	UAGCUUAUCAGUCUGAUGUUGA
Mis-2	UAGCUUAUCAGUCUCAUGUUGA
Mis-4	UAGCUUAUCAGUCUCAUCUUGA
miRNA-145	GUCCAGUUUUCCCAGGAUCCCU
pDNA1	GTTGATGAAGCTGT
H	TCAACATCAGTCTGATAAGCTATGACGAACTAGTTGATGAAGCT GTGTCATAGCTTATCAGA

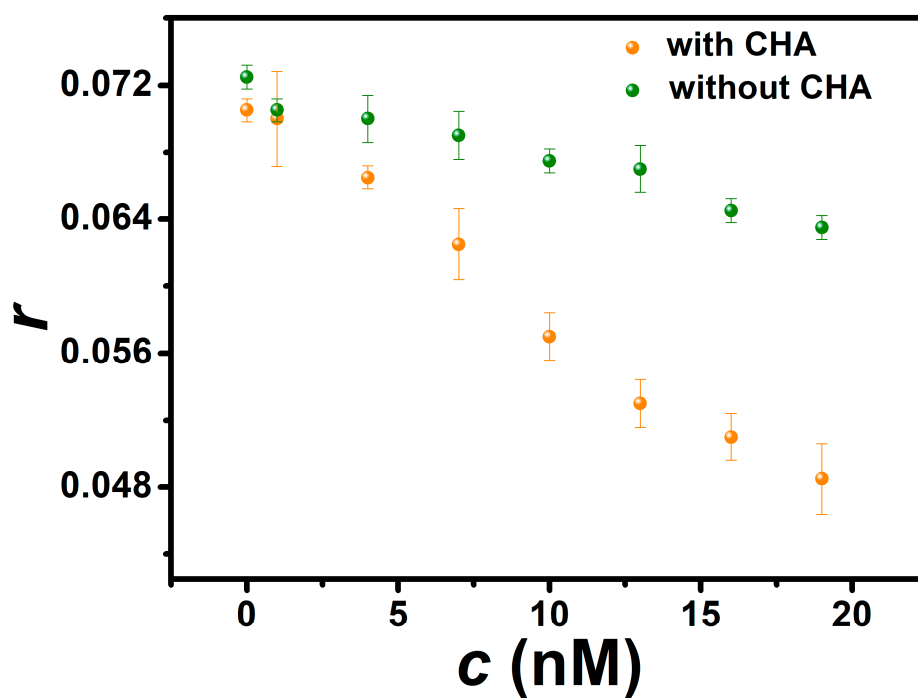
**Table S2.** The reagents used in preparation of the DSN.

Name	Concentration ( $\mu\text{M}$ )	Name	Concentration ( $\mu\text{M}$ )
T1	0.15	As-A	0.30
P1	0.23	As-B	0.46
T2	0.23	initiator	0.075
P2	0.35	linker DNA	0.2
		SA	0.1

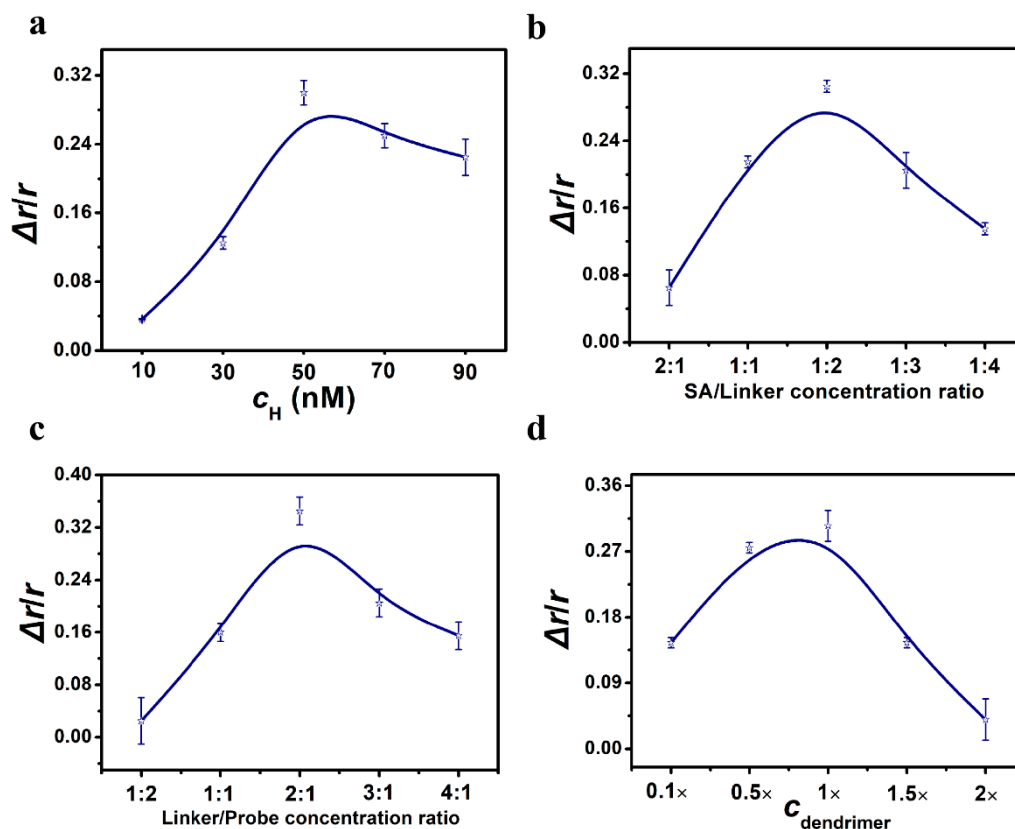
The concentrations shown in Table S2 are abbreviated as 1 $\times$ .



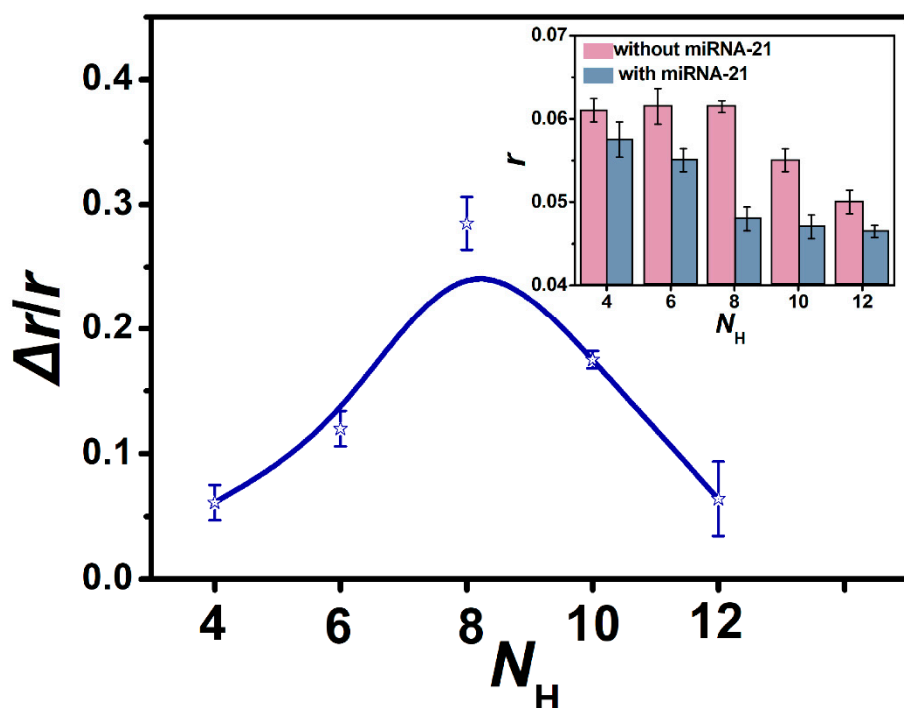
**Figure S1.** Schematic illustration of DNA dendrimer enhanced FA for miRNA-21 detection without CHA. (Double arrows represent the possible rotational contributions to the FA change).



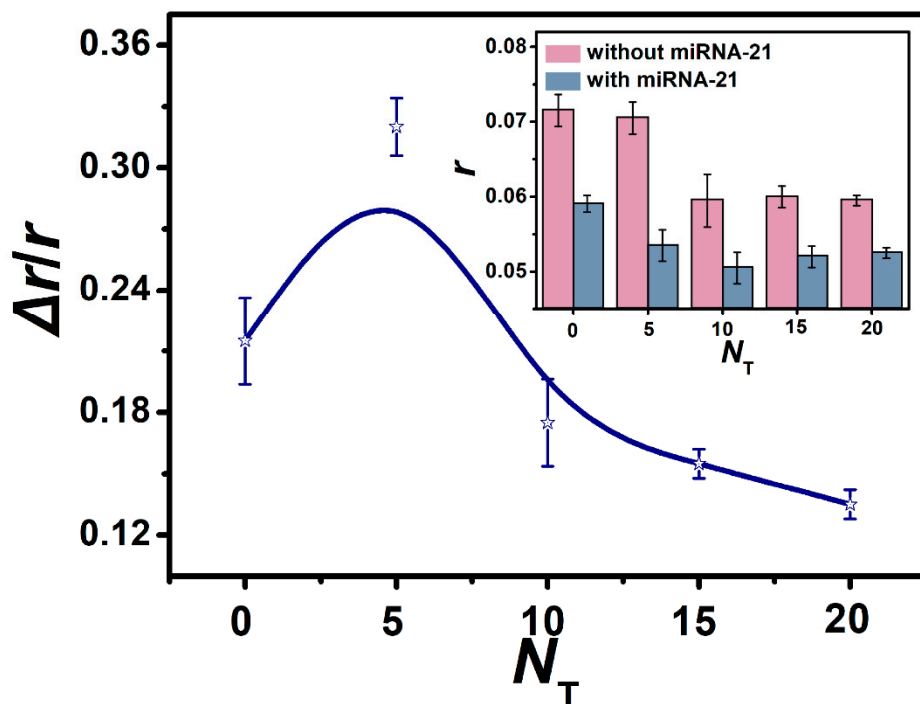
**Figure S2.** Function of CHA. Fluorescence anisotropy ( $r$ ) in the absence and presence of CHA after the addition of miRNA-21 with different concentrations. Concentrations: H1, H2, 50 nM; DNA dendrimer-pDNA, 1x; miRNA, 9 nM.



**Figure S3.** Optimization of experimental conditions. (a) FA changes at different concentrations of H1 and H2. Concentrations: DNA dendrimer-pDNA, 1x; miRNA-21, 9 nM. (b) Influence of the linker DNA/SA concentration ratio on FA response of amplified mass biosensor. Concentrations: H1, H2, 50 nM; miRNA-21, 9 nM. (c) Influence of the linker DNA/Probe concentration ratio on FA response of amplified mass biosensor. Concentrations: H1, H2, 50 nM; miRNA-21, 9 nM. (d) FA response at the different concentration of DNA- dendrimer. Concentrations: H1, H2, 50 nM; miRNA-21, 9 nM.



**Figure S4.** FA ( $r$ ) in the absence and presence of the miRNA-21 with different bases number ( $N_H$ ) of toehold domain of H1. 4, 6, 8, 10, 12 represent  $N_H$  is 4, 6, 8, 10, 12. Concentrations: H1-1, H2-1, H1-2, H2-2, H1, H2, H1-3, H2-3, H1-4, H2-4, 50 nM; DNA dendrimer-pDNA, 1 $\times$ ; miRNA-21, 9 nM.



**Figure S5.** FA ( $r$ ) in the absence and presence of the miRNA-21 with different bases number ( $N_T$ ) of T spacer on linker DNA (Linker, Linker 1, Linker 2, Linker 3, Linker 4). 0, 5, 10, 15, 20 represent  $N_T$  is 0, 5, 10, 15, 20. Concentrations: H1, H2, 50 nM; miRNA-21, 9 nM.

**Table S3.** Comparison of different sensors for miRNA-21 assay.

Method	Materials	Instrument	Line range	LOD	Ref
Fluorescent sensor	apurinic/apyrimidinic endonuclease 1,DNA	Spectrofluorimeter	2.5-40 nM	0.25 nM	[1]
Fluorescent sensor	AuNPs,DNA N-methyl	Spectrofluorimeter	0-10 nM	10 pM	[2]
Fluorescent sensor	mesoporphyrin IX,DNA, lambda exonuclease	Spectrofluorimeter	0-250 nM	1.4 pM	[3]
colorimetric sensor	Graphene/gold-nanoparticle, DNA	UV/vis spectrophotometer	10 nM-0.9 $\mu$ M	3.2 nM	[4]
Electrochemical sensor	MBs,DNA, nuclease	glucose meters	10-200 pM	1.8 pM	[5]
Lateral flow biosensor	AuNPs,DNA	–	10 pM-10 nM	0.1 nM	[6]
Lateral flow biosensor	FMs,DNA	smartphone	0.5-16 nM	230.6 pM	[7]
Ratiometric Fluorescent sensor	ZnO-NH <sub>2</sub> , DNA	spectrofluorometer	0.5-150 nM	83 pM	[8]
glucose meter sensor	DNA, Exo T	personal glucose meter	5-150 nM	3.65 nM	[9]
Fluorescent sensor	DNA,SA	spectrofluorometer	1-19 nM	52 pM	This work

**Table S4.** Recovery experiment for the detection of miRNA-21 in human serum based on the linear equation of the human serum.

Sample	Add (nM)	Found (nM)	Recovery (%)	RSD(%) (n=3)
1%Human serum	2	2.1±0.4	91.8-116.3	0.9
	10	10.9±0.8	100.6-114.1	1.8
	18	20.8±2.1	103.4-125.1	5.4

## References

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