Supplementary Information

For

A New AgNC Fluorescence Regulation Mechanism Caused by Coil DNA and Its Application in Constructing Molecular Beacons with Low Background and Large Signal Enhancement

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Materials. DNA samples (purchased from Sangon Biotechnology Co., Ltd. Shanghai, China) were dissolved in phosphate buffer (PB: 20 mM Na_2HPO_4/NaH_2PO_4, 10 mM $Mg(Ac)_2$, pH 7.5) and quantified by UV absorbance at 260 nm. The extinction coefficients were obtained by summing extinction coefficients of individual bases in each sequence: $\varepsilon(dA) = 15400\ M^{-1}cm^{-1}$, $\varepsilon(dG) = 11500\ M^{-1}cm^{-1}$, $\varepsilon(dC) = 7400\ M^{-1}cm^{-1}$ and $\varepsilon(dT) = 8700\ M^{-1}cm^{-1}$. Sodium borohydride ($NaBH_4$) and silver nitrate ($AgNO_3$) were bought from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were analytically pure and used without pretreatment. Milli-Q water (specific resistance of 18.2 MΩ) was used throughout.

Preparation of DNA-templated AgNC. AgNCs were obtained according to previous references (Teng et al. 2016). Briefly, 10 μM $AgNO_3$ was mixed with 1 μM DNA template in PB solution. After reacting at room temperature for 1 hour, 10 μM fresh $NaBH_4$ was added with vigorous shaking for 2 min, and then the reaction solution was incubated in dark for 6 hours before use. In this work, AgNC was quantified by the concentration of DNA template.

Characterizations of DNA-templated AgNC. Cary 500 Scan UV–Vis–NIR spectrophotometer (Varian) and Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France) were used to collect the UV–Vis absorption spectroscopy and fluorescence spectra data. Slit widths of fluorescence spectrophotometer for excitation and emission were both 10 nm. Transmission electron microscopy (TEM) images were recorded on a JEM-2100F high-resolution transmission electron microscope operating at 200 kV. The fluorescence lifetimes of AgNC were performed by a FLS920 spectrofluorometer and measured at emission peaks at 570 nm and 630 nm with excitation wavelength at 375 nm.

Quantum yields of the AgNC. Quinine sulfate in 0.1 M H_2SO_4 was chosen as the standard. The quantum yield of quinine sulfate was 0.54 at 360 nm. According to the formula below, the quantum yields of A_{20}-C55-NC were calculated.

$$Q=(Q_R \cdot I \cdot A_R \cdot n^2) / (I_R \cdot A \cdot n_R^2)$$

(In this formula, Q is the quantum yield, I is the measured integrated emission intensity, n is the refractive index, and A is the optical density.)

Target DNA analysis. To detect target DNA, the prepared AgNC probes were diluted to 100 nM and incubated with different amounts of target DNA at room temperature for 2 h, followed by fluorescence measurement.

Supplementary Results

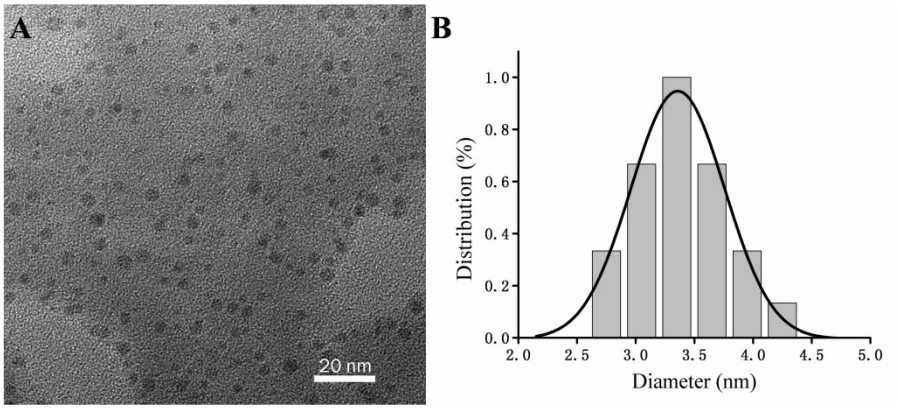
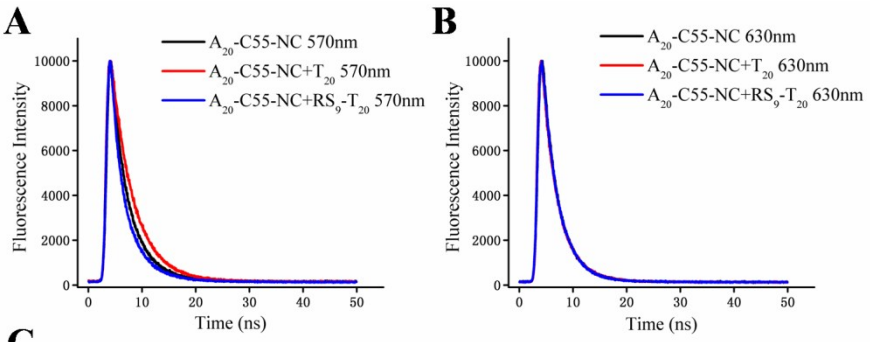


Fig. S1 TEM image (A) and size distribution (B) of A_{20}-C55-NC.



C

AgNC \ $\bar{\tau}$ (ns)	FL_{570}	FL_{630}
A_{20}-C55-NC	3.33	2.89
A_{20}-C55-NC+T_{20}	3.76	2.93
A_{20}-C55-NC+RS_9-T_{20}	3.17	2.94

Fig. S2 (A and B) The variation of fluorescence emission decays measured at 570 nm (A) and 630 nm (B) when A_{20}-C55-NC hybridizing with T_{20} and RS_9-T_{20}, respectively. (C) Average fluorescence lifetimes of AgNC measured at 570 nm and 630 nm, respectively.

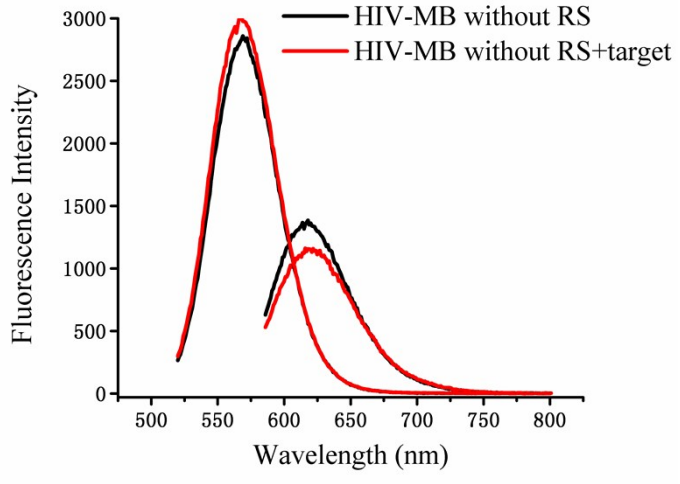


Fig. S3 Fluorescence variation of HIV-MB without random sequence before and after hybridizing with HIV target DNA.

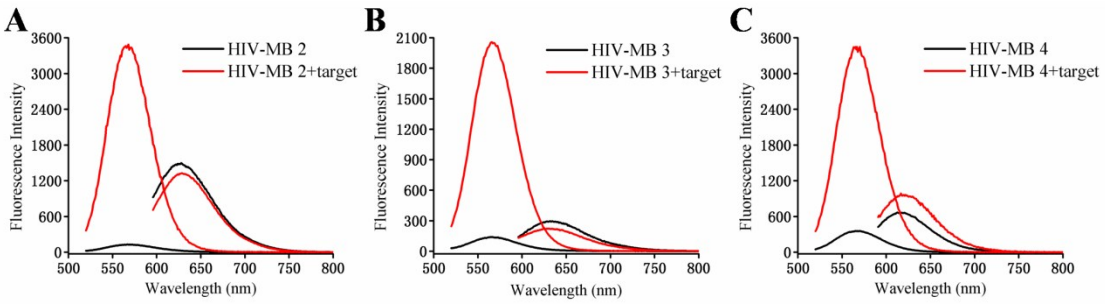


Fig. S4 Fluorescence spectra of HIV-MB 2 (A), HIV-MB 3 (B) and HIV-MB 4 (C) before and after hybridizing with target DNA.

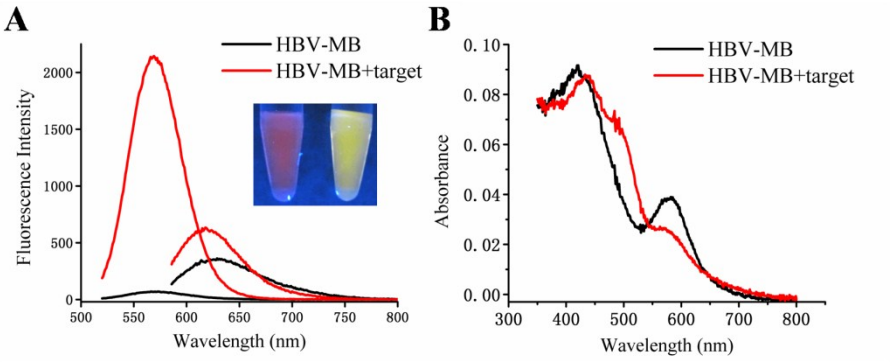
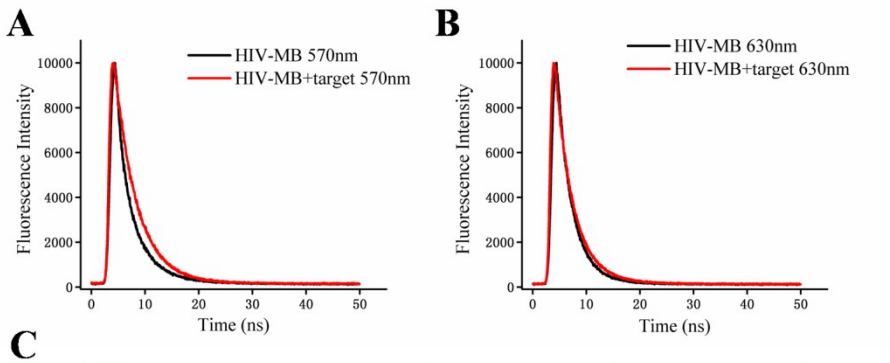


Fig. S5 (A) Fluorescence and (B) UV-Vis absorption spectra HBV-MB before and after hybridizing

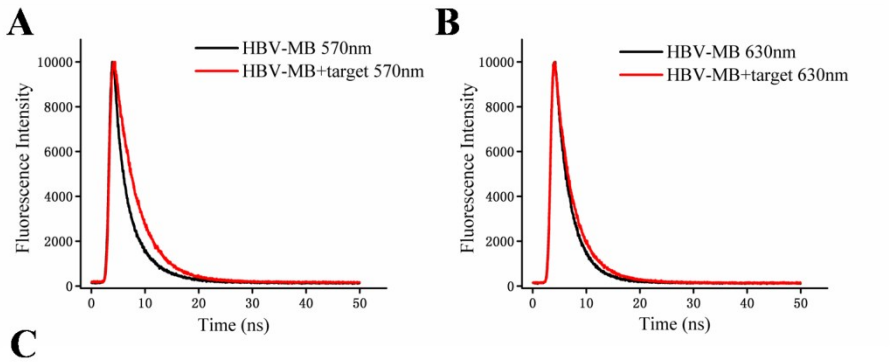
with HBV target DNA. Photograph: Fluorescence color of AgNC solution before (left) and after (right) the addition of target DNA under UV irradiation.



C

AgNC / $\bar{\tau}$ (ns)	FL_{570}	FL_{630}
HIV-MB	3.59	2.96
HIV-MB+HIV Target	3.96	3.17

Fig. S6 (A and B) The variation of fluorescence emission decays measured at 570 nm (A) and 630 nm (B) before and after HIV-MB hybridizing with HIV target, respectively. (C) Average fluorescence lifetimes of HIV-MB measured at 570 nm and 630 nm, respectively.



C

AgNC / $\bar{\tau}$ (ns)	FL_{570}	FL_{630}
HBV-MB	3.43	2.95
HBV-MB+HBV Target	4.12	3.25

Fig. S7 (A and B) The variation of fluorescence emission decays measured at 570 nm (A) and 630 nm (B) before and after HBV-MB hybridizing with HBV target, respectively. (C) Average fluorescence lifetimes of HBV-MB measured at 570 nm and 630 nm, respectively.

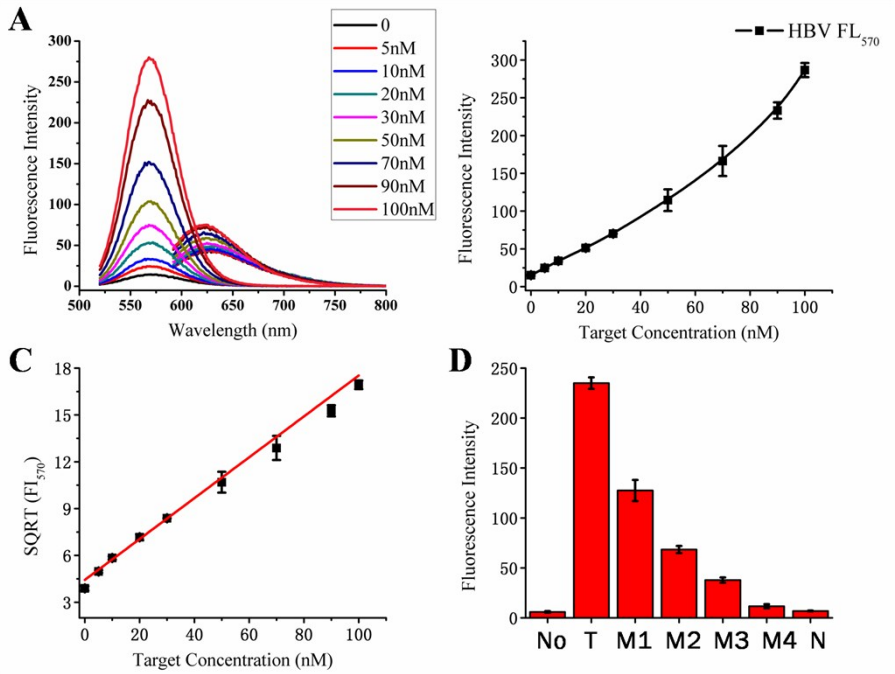


Fig. S8 (A) Fluorescence variation of HBV-MB mixing with different concentrations of HBV target. (B) Calibration curve of FL_{570} versus target concentration. (C) Concentration curve of FL_{570} of HBV-MB versus different amounts of HBV target. (D) Selectivity of the HBV-MB for analyzing target DNA. No: HBV-MB sequence only; T: added with 100 nM target sequence; M1-M4: added with 100 nM sequences had 1–4 base variations to target DNA, respectively; N: added with 100 nM irrelative gene sequence to target DNA. Error bars indicate the standard deviations of three measurements of independent samples for each concentration of HBV target. DNA template concentration of HBV-MB is 100nM.

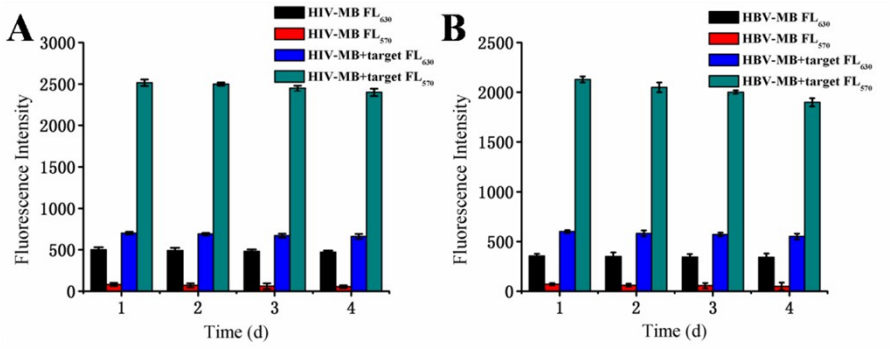


Fig. S9 Fluorescence intensity variations of HIV-MB (A) and HBV-MB (B) before and after hybridizing with targets in 4 days. Error bars indicate the standard deviations of three measurements of independent samples for each day.

Oligo name	DNA Sequence (5'→3')
A_{20}-C55	AAAAAAAAAAAAAAAAAAAACCCCCTAATTCCCCC
T_{20}	TTTTTTTTTTTTTTTTTTTT
TC-T_{20}	TCTTTTTTTTTTTTTTTTTT
G_2-T_{20}	GGTTTTTTTTTTTTTTTTTT
A_2-T_{20}	AATTTTTTTTTTTTTTTTTT
C_2-T_{20}	CCTTTTTTTTTTTTTTTTTT
RS_5-T_{20}	AATCCTTTTTTTTTTTTTTTTTT
RS_7-T_{20}	TTAATCCTTTTTTTTTTTTTTTTTT
RS_9-T_{20} ($C_4T_3A_2$-T_{20})	CCTTAATCCTTTTTTTTTTTTTTTTTT
G_7T_2-T_{20}	TGGGGTGGGTTTTTTTTTTTTTTTTTT
A_4T_5-T_{20}	TATTAATTATTTTTTTTTTTTTTTTTT
$A_3G_2C_2T_2$-T_{20}	ATCGATCGATTTTTTTTTTTTTTTTTT
C_9-T_{20}	CCCCCCCCCTTTTTTTTTTTTTTTTTT
A_5T_4-T_{20}	ATATATATATTTTTTTTTTTTTTTTTT
RS_{12}-T_{20}	CCCTTAATCCCCTTTTTTTTTTTTTTTTTT
RS_{15}-T_{20}	CCCCCTTAATCCCCCTTTTTTTTTTTTTTTTTT
RS_{18}-T_{20}	CCCCCCTTAATTCCCCCCCTTTTTTTTTTTTTTTTTT
$G_{11}T_3$-T_{20}	TGGGGTGGGGTGGGTTTTTTTTTTTTTTTTTT
$G_{15}T_3$-T_{20}	GGGGTGGGGTGGGGTGGGTTTTTTTTTTTTTTTTTT

HIV-MB	**CCTTAATCCTTAGAGAGTCAGTGTGGAAAATCTCTAAACCCCCTAATTCCCCC**
HIV-MB 2	**ATCGATCGATTAGAGAGTCAGTGTGGAAAATCTCTAAACCCCCTAATTCCCCC**
HIV-MB 3	**TGGGGTGGGTTAGAGAGTCAGTGTGGAAAATCTCTAAACCCCCTAATTCCCCC**
HIV-MB 4	**TCTTAATTATTAGAGAGTCAGTGTGGAAAATCTCTAAACCCCCTAATTCCCCC**
HIV target	**TAGAGATTTTCCACACTGACT**
HIV M1	**TAGAGATTGTCCACACTGACT**
HIV M2	**TAGAGATTGTCCACACGGACT**
HIV M3	**TAGAGATTGTCCTCACGGACT**
HIV M4	**TAGATATTGTCCTCACGGACT**
N	**AGGTAGTAGATTGTATAGTTA**
HBV-MB	**CCTTAATCCTTTTCAGACATCATCCATATAACTGAAAACCCCCTAATTCCCCC**
HBV target	**TTTCAGTTATATGGATGATGT**
HBV M1	**TTTCAGTTATATTGATGATGT**
HBV M2	**TTTCATTTATATTGATGATGT**
HBV M3	**TTTCATTTATATTGATTATGT**
HBV M4	**TTTCATTTTTATTGATTATGT**

Table S1. DNA strands used in this study.

AgNC	Quantum yield of 570 nm (Q_Y)	Quantum yield of 630 nm (Q_R)	Quantum yield of total (Q_t)
A_{20}-C55-NC	0.0159	0.0249	0.0408
A_{20}-C55-NC+T_{20}	0.1686	0.0221	0.1907
A_{20}-C55-NC+RS_9-T_{20}	0.0007	0.0193	0.0200

Table S2. Quantum yields of A_{20}-C55-NC before and after hybridizing with T_{20} and RS_9-T_{20}.