Supporting Information

A sandwich sensor based on imprinted polymer and aptamer for highly-specific double recognition of virus

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DLS Analysis of SiO_2-NH_2, CS, MIP and NIP

The sizes of SiO_2-NH_2, CS, MIP and NIP have been repeated using DLS, and the result is shown in **Fig.S1**. From the results we can see, the particle size of SiO_2-NH_2 is about 235 nm, the particle size of CS is about 253 nm, and the particle size of MIP and NIP is about 305 nm.

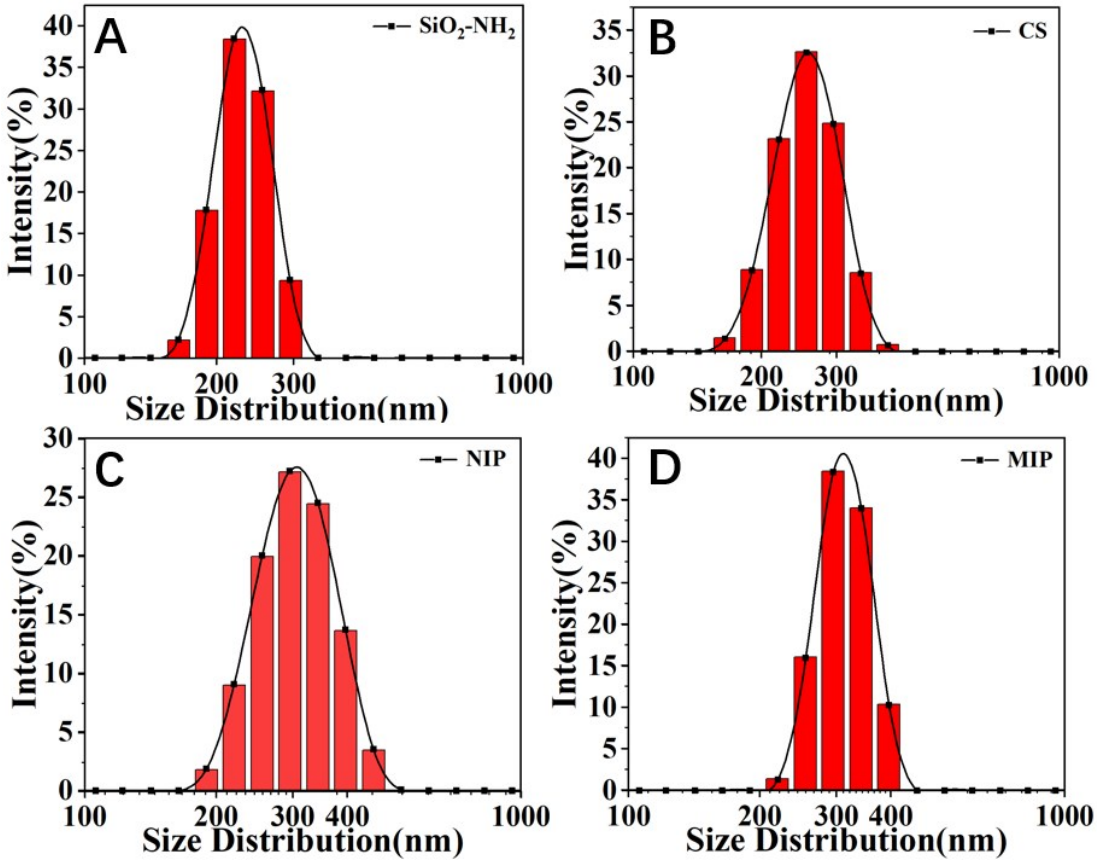

Fig. S1 Size Distribution of SiO_2-NH_2 (A), CS (B), NIP (C), MIP (D)

Optimization of the assay conditions

To obtain a sensitive and practical assay for detection of HBV, various factors that could potentially affect the detection efficiency were optimized, including (a) the dosage of MIP, (b) The ratio of MIP and SiO_2@Apt, (c) pH, (d) incubation temperature, and (e) incubation time.

The influence of the ratio of MIP and SiO_2@Apt on the test results was

investigated when other conditions were kept constant (**Fig. S2A**). It can be seen that when the ratio of MIP to SiO_2@Apt is 1:2, the imprinting factor is the highest and the detection effect is the best. This can be explained as: at this time, the HBV adsorbed on the MIP is the most, and can also be bound by the aptamer in the highest amount, causing the largest amplitude signal change.

When the concentration of HBV was 0.1 $nmol \cdot L^{-1}$, the mass of MIP and SiO_2@Apt was investigated from 20 $ng \cdot mL^{-1}$ to 120 $ng \cdot mL^{-1}$ to achieve the best detection effect and the maximum IF. As shown in **Fig. S2B**, the results showed that the IF obviously increased with rising mass of particles, and achieved the most at 80 $ng \cdot mL^{-1}$. This may be because the content of HBV in the system was excessive relative to the particles within this range. As the concentration of particles increases gradually, more HBV was identified by imprinting sites and aptamer, resulting in a more noticeable changes in signal. When the concentration of particles exceeds 80 $ng \cdot mL^{-1}$, the ΔI_{RLS} and IF decrease with the increase of particles concentration attribute to the presence of more unsaturated MIPs particles in the system, or not enough aptamers to bind, which may be because the virus that can be combined by the imprinting sites has reached a maximum, and non-specific combination may also increase.

The effect of pH in a range of 5.0 to 9.0 was studied (**Fig. S2C**). The results showed that the value of IF obviously increased with rising pH when the pH was lower than 7.4, and then declined with the increase of pH after exceeding 7.4. This may be because the amide bond between the target molecule and MIP or aptamer and SiO_2-NH_2 tends to be stable at relatively neutral pH value, thus binding to the target molecule. Excessive acid or alkaline will cause the hydrolysis of the amide bond to destroy the interaction between HBV and MIP or SiO_2-NH_2 and the aptamer. On the other hand, when pH=7.4, biomolecules prepared under physiological conditions,

such as aptamers and HBV, can maintain relatively high activity. Therefore, the pH value of the combined reaction is selected as 7.4.

The effect of temperature on the detection was evaluated at 20 ^{o}C to 40 ^{o}C, respectively (**Fig. S2D**). The rebinding tests were performed at different temperature, and then ΔI_{RLS} and IF were measured. The best detection response occurred at 25 ^{o}C. This may lead a worse strength of the binging force between the HBV and MIP or aptamer at too high or too low temperature.

By changing the incubation time from 0 min to 60 min, the combination kinetics of HBV by MIPs or NIPs (**Fig. S3A**) and by SiO_2-NH_2@Apt (**Fig. S3B**) was measured. Under optimized conditions, after the same concentration of MIPs and NIPs were incubated with HBV for 1 h, and the combination of HBV by MIP or NIP reaches the highest value in about 20 min. In order to ensure the full combination of HBV by MIP, 1 h was still selected as the incubation time of first step. The second step of recognition was performed with SiO_2-NH_2@Apt. A rapid increase in the ΔI_{RLS} was observed for MIPs, and the reaction process almost reached highest point after 30 min for both MIPs and NIPs respectively, and then tended to be equilibrium with the time extension. However, the maximum combination capacity of MIPs is about 7.2 times that of NIPs, and the effect is significantly improved. It was reasonable to assume that this combination equilibrium was due to the smaller diffusion barrier with the thin imprinting layer and specific recognition was improved greatly due to the dual recognition of MIPs and aptamers. Thereafter, 30 min was selected to be the incubation time.

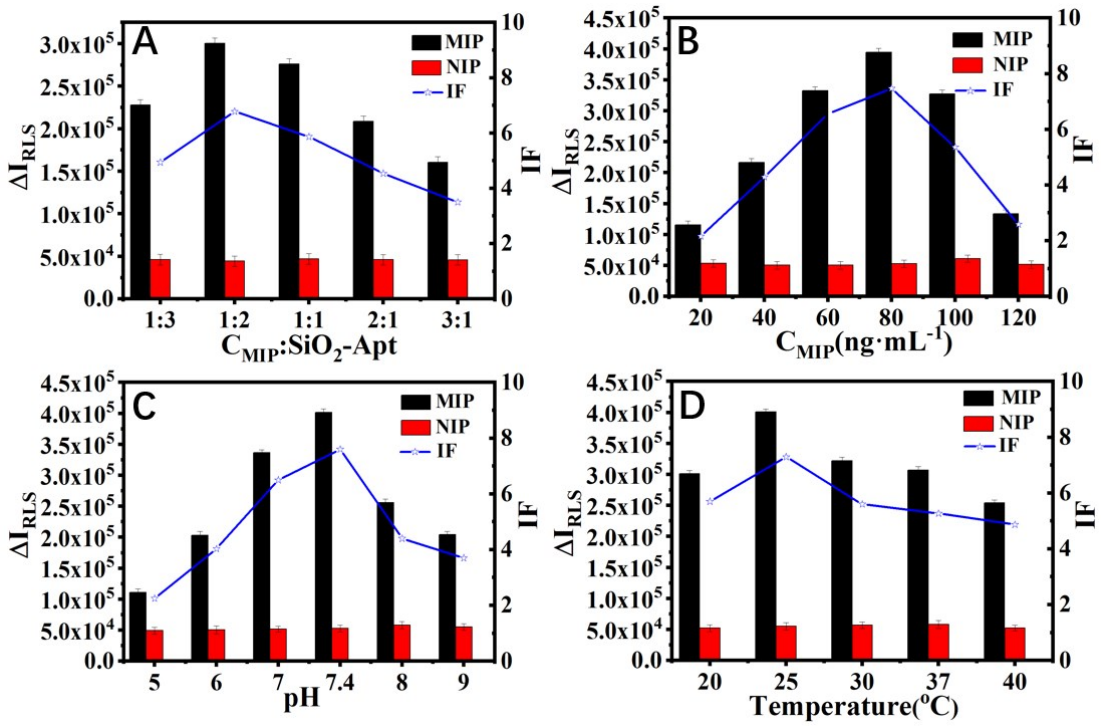

Fig. S2 Effect of (A) the dosage ratio of MIPs and SiO_2@Apt, (B) the total dosage of MIPs and SiO_2@Apt, (C) pH and (D) incubation temperature on the IF of the MIP and NIP. Error bars represent standard deviations for 3 parallel measurements. S2(A) Conditions: TEOS: 2.5 μL·mL^{-1} (98%); Apt: 33 nM; nanospheres dosage: C_{MIP}=100 ng·mL^{-1}, $C_{SiO2\text{-}Apt}$=300 ng·mL^{-1}, 200 ng·mL^{-1}, 100 ng·mL^{-1}, 50 ng·mL^{-1}, 33.3 ng·mL^{-1}; concentration of HBV: 0.1 nM; pH: 7.4; temperature: 25 ℃; incubation time: 60 min; S2(B) Conditions: TEOS: 2.5 μL·mL^{-1} (98%); Apt: 33 nM; C_{MIP} : $C_{SiO2\text{-}Apt}$=1:2; concentration of HBV: 0.1 nM; pH: 7.4; temperature: 25 ℃; incubation time: 60 min; S2(C) Conditions: TEOS: 2.5 μL·mL^{-1} (98%); Apt: 33 nM; C_{MIP} : $C_{SiO2\text{-}Apt}$=1:2=80:160 (ng·mL^{-1}); concentration of HBV: 0.1 nM; temperature: 25 ºC; incubation time: 60 min; S2(D) Conditions: TEOS: 2.5 μL·mL^{-1} (98%); Apt: 33 nM; C_{MIP} : $C_{SiO2\text{-}Apt}$=1:2=80:160 (ng·mL^{-1}); concentration of HBV: 0.1 nM; pH: 7.4; incubation time: 60 min.

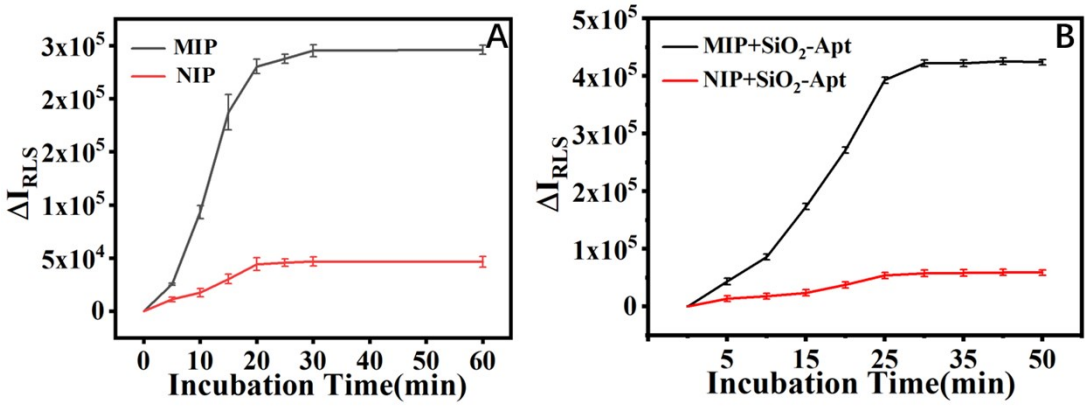

Fig. S3 (A) Effect of incubation time of the MIP and NIP to HBV on the ΔI_{RLS}; (B) Effect of incubation time of the SiO_2-Apt to HBV on the ΔI_{RLS}. Error bars represent standard deviations for 3 parallel measurements. (Conditions: TEOS: 2.5 μL·mL^{-1} (98%); Apt: 33 nM; C_{MIP}: $C_{SiO2\text{-}Apt}$=1:2=80:160 (ng·mL^{-1}); concentration of HBV: 0.1 nM; pH: 7.4; temperature: 25 ^{o}C)

Specificity test of the aptamers

In addition to imprinted polymers, the specificity of aptamers is also an important criterion for evaluating detection performance. In order to study the specificity of aptamers, non-target virus H5N1 aptamer (Apt-H5N1) and completely mismatched aptamer sequence (Apt-W) were used to construct sensors under the same conditions and incubated to evaluate their detection performance.

Preparation of SiO_2@Apt: 50 μL 1×10^{-7} M Apt (Apt-HBV, Apt-H5N1, Apt-W) was activated with 25 μL EDC (0.1 M) and 25 μL NHS (0.025 M) for 1 h, then 50 μL 25 $mg\cdot mL^{-1}$ SiO_2-NH_2 was added and incubated at 37 ^oC for 6 h. The resulting solution was centrifuged to remove excess aptamer, and then was redispersed in 50 μL water to be stored at 4 ^oC

First, the assembly of the aptamer was characterized, and the result is shown in **Fig. S4**, the decrease of Zeta potential indicates that all three aptamers are modified on SiO_2 nanoparticles. After incubation with HBV for a period of time, the sensor corresponding to the three aptamers has obvious differences in the combination of the target virus. The sensor constructed by the target virus aptamer (Apt-HBV) and MIP has the best detection performance as well as an obvious secondary enhancement of resonance light intensity, while the sensor constructed by Apt-H5N1 or Apt-W has no obvious change in resonance light intensity and low imprinting factor. Moreover, due to less non-specific combination of HBV, the change of resonance light intensity of NIP was not obvious (**Fig. S5**). The above results indicate that aptamers play an important role in improving sensor specificity.

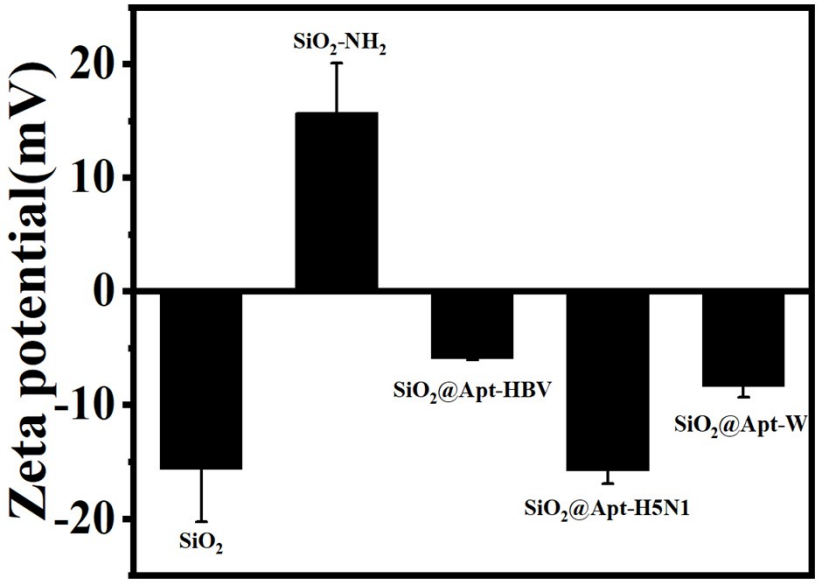

Fig. S4 Zeta potential of SiO_2, SiO_2-NH_2, SiO_2@Apt-HBV, SiO_2@Apt-H5N1 and SiO_2@Apt-W. Error bars represent standard deviations for 3 parallel measurements.

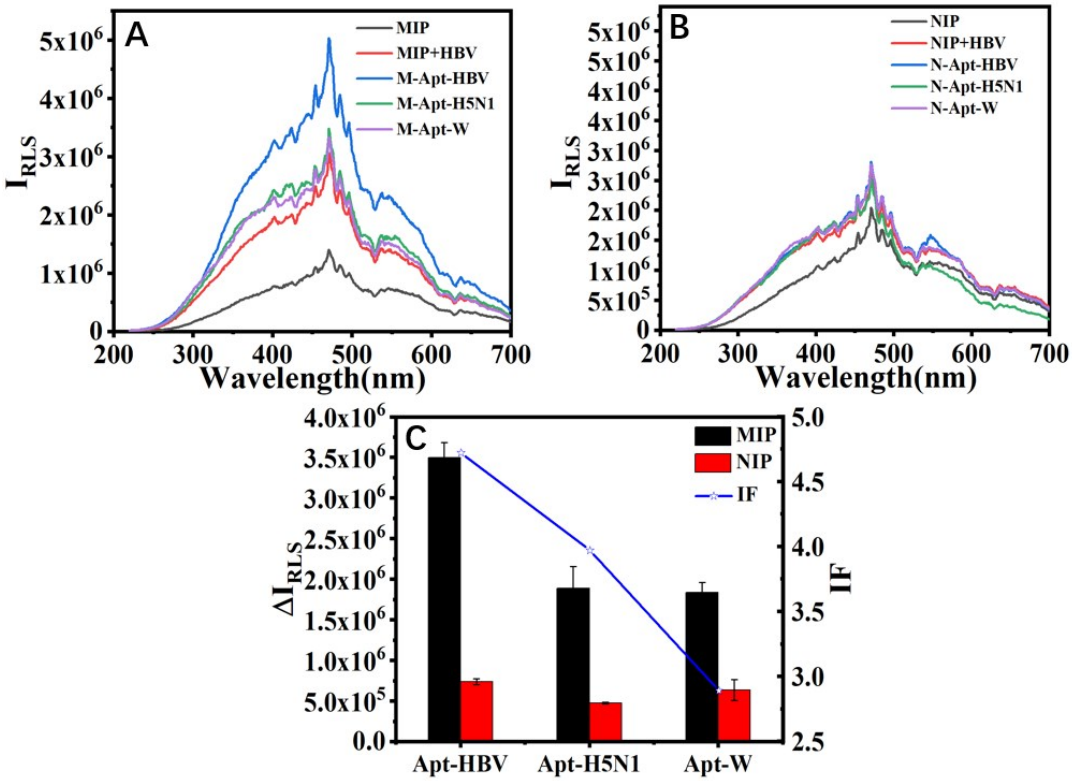

Fig. S5 Effect of different aptamers on the I_{RLS} of (A) MIP and (B) NIP sensor; (C) Effect of different aptamers on the ΔI_{RLS} and IF. Error bars represent standard deviations for 3 parallel measurements. (Conditions: TEOS: 2.5 $\mu L \cdot mL^{-1}$ (98%); Apt: 33 nM; C_{MIP}: $C_{SiO2\text{-}Apt}$=1:2=80:160 ($ng \cdot mL^{-1}$); concentration of HBV: 3 nM; pH: 7.4; temperature: 25 ^{o}C; incubation time: 60 min)