SUPPORTING INFORMATION

Chemiluminescent Labels Released from Long Spacer Arm-Functionalized Magnetic Particles: A Novel Strategy for Ultrasensitive and Highly Selective Detection of Infectious Pathogens

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Author Contributions

H. Y. and W. L. contributed equally to this work.

CL Detection of SA-AP and MPs

Chemiluminescent (CL) intensity was detected by a 2030 Multilabel Plate Reader (PerkinElmer). Figure S1 indicated that CL intensity in the system of AP and AMPPD was significantly limited by the inner filter-like effect arising from excess dark magnetic particles (MPs).

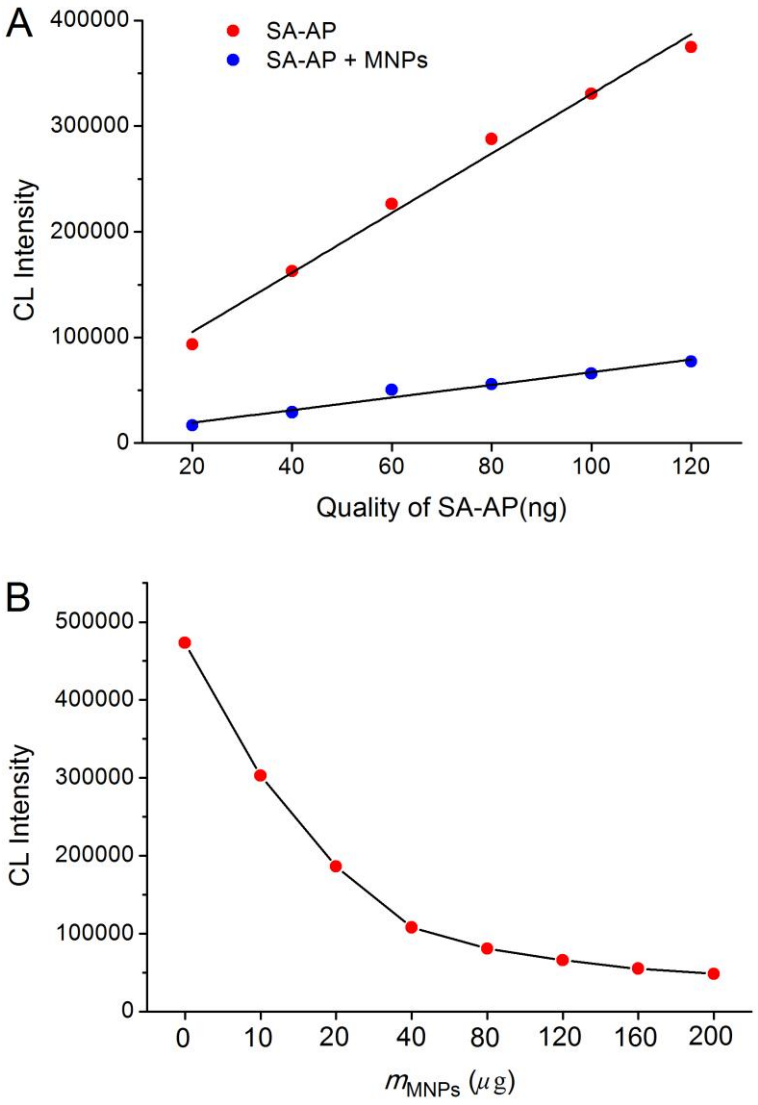

Figure S1. (A) CL intensity versus the amount of streptavidin-alkaline phosphatase (SA-AP) without MPs (red circles) and with 200 μg of MPs (blue circles). (B) CL intensity versus the amount of MPs with 160 ng of SA-AP. The detection procedure was carried out as described in the experimental section.

Primer and Probe Design.

The primers and probe for HBV detection were designed using Premier Primer 5 software (PREMIER Biosoft). The sequence information was listed in Table S1.

Table S1. Oligonucleotide sequences of the primers and probe used for the detection of HBV.

Target	**Sequence Name**	**Sequence (5′–3′)**	**Amplicon Size**
Primer	*X*_forward	CCTCTACCGTCCCCTTCTTCA	126 bp
	*X*_reverse	ACGTGCAGAGGTGAAGCGAAG	
Probe	*X*_Probe	CACTTCGCTTCACCTCTGCACGTA-$(T)_{15}$-NH_2	

Characterization

(a) *TEM and SEM Images* The morphology and particle size of the MPs samples were determined by TEM with a JEM2200CX transmission electronic microscope and by SEM with a Hitachi-S100 scanning electron microscope. But Figure S2A suggested that no obvious distinction was found between before and after CMG modification.

(b) *Dynamic Light Scattering Analysis*. The hydrodynamic sizes of the MPs samples were determined by dynamic light scattering (DLS) using a nano Zetasizer Nano ZSP (Marlven, Ltd). Figure S2B showed that the average hydronamic size was CMG-MPs 677.9 nm in MPs and 772.2 nm in CMG-MPs.

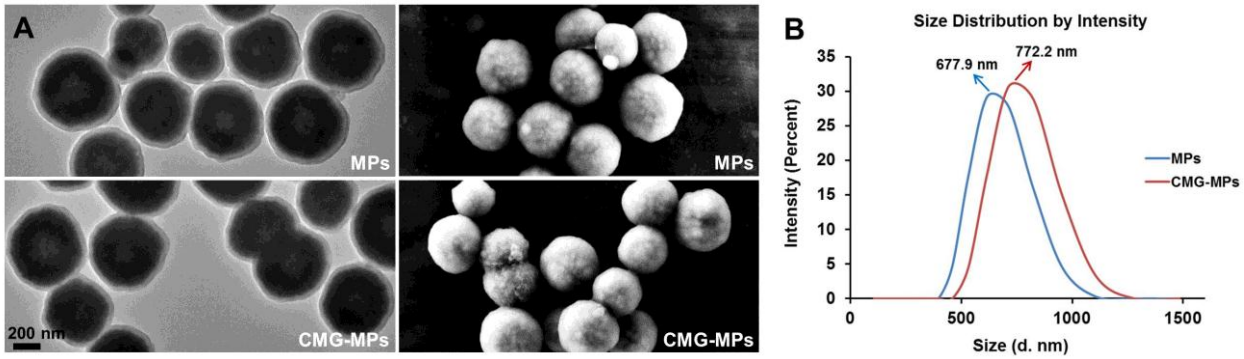

Figure S2. (A) TEM image (a) and SEM image (b). (B) The hydrodynamic sizes by DLS.

Gel Electrophoresis Analysis of HBV Amplicons

The PCR products containing HBV amplicons were analyzed by gel electrophoresis. Figure S3 presented clear bands with a size of 126 bp, which proved the successful formation of the biotinylated HBV amplicons and the lack of formation of nontarget amplicons.

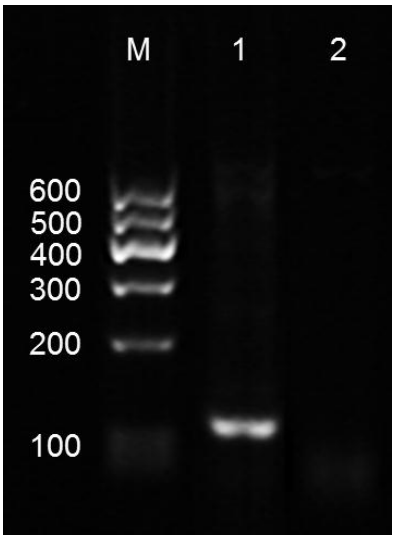

Figure S3. Image of the gel after electrophoresis of PCR products showing the target band of the biotinylated HBV amplicon. Lane M: DNA markers (100–600 bp). Lane 1: the biotinylated HBV amplicon (126 bp) is visible. Lane 2: blank control; the HBV DNA template was replaced with deionized water. The PCR assay was carried out as described in the experimental section.

Pre-Experiment for DNase Degradation-Based CL Detection

In the pre-experiment, 15 U of DNase I were employed to degrade DNA for different time periods (10–30 min) according to the manufacturer's instructions, but Figure S4 indicated no obvious difference in CL intensity.

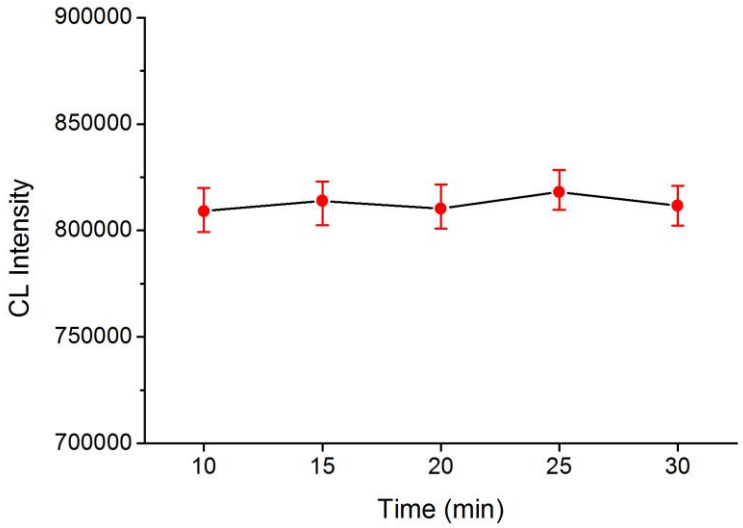

Figure S4. CL intensity versus DNase degradation time. The detection procedure was carried out as described in the experimental section. Error bar: standard deviation of three independent measurements.

Optimization of Assay Parameters for CMPs-Based CL Detection

The concentration of DNA-capture probes, hybridization temperature, and hybridization time for carboxylated MPs (CMPs)-based HBV detection were investigated by monitoring the changes in CL intensities. Figure S5 illustrated that the optimal conditions for CMP-based HBV detection were 2 μM probe concentration, 50°C hybridization temperature, and 30 min hybridization time.

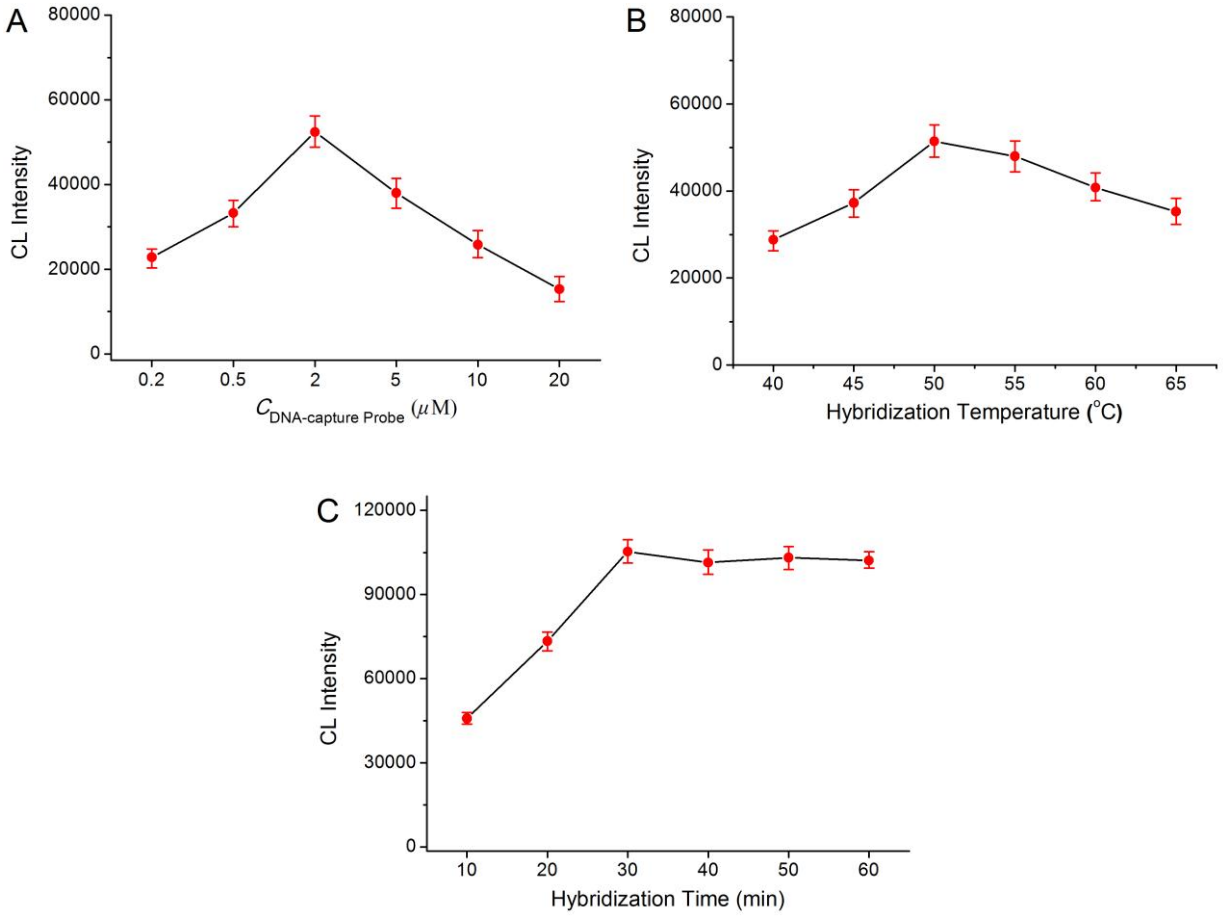

Figure S5. Optimization of assay parameters for the detection of HBV using CMPs. CL intensity versus the concentration of DNA-capture probe (A), hybridization temperature (B), and hybridization time (C). The detection procedure was carried out as described in the experimental section. Error bar: standard deviation of three independent measurements.