Supporting Information

Sensitive chemiluminescence immunoassay for *E. coli* O157:H7 detection with signal dual–amplification using glucose oxidase and laccase

Yun Zhang,[†,‡,§] Chen Tan, [†,§] Ruihua Fei,[†,‡] Xiaoxiao Liu,[†,‡] Yuan Zhou,[†,‡] Jing Chen,[†] Huanchun Chen,[†] Rui Zhou,[†] Yonggang Hu [*] [†,‡]

[†] *State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China*

[‡] *College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*

Optimization of Immunoassay Conditions

Lacasse significantly enhanced the CL signal of a luminol–H_2O_2 system in a highly basic medium. This enhancement may be due to the fact that the basic pH is beneficial to the decomposition pattern of the key intermediate alpha-hydroxy-hydroperoxide to ground-state phthalate and facilitates high chemiluminescence signals. This decomposition reaction was unique, depended only on pH of the system[1], was presumed to be the rate-determining step of luminol CL.[2] The effect of pH on the variance in CL ratio of I/I_0 (where I and I_0 are the CL signals with and without *E. coli* O157:H7, respectively) was investigated by varying the pH from 10.3 to 12.6. Figure S1A shows a significant increase in I/I_0 increased at pH 11.0 but a decrease with further increased pH. The pH dependence of light emission may be due to the effect of enzyme inactivation at pH > 11.0. After H_2O_2 formation, the pH of the luminol–supernatant–laccase mixture was adjusted to 11.0 using 0.1 mol L^{-1} NaOH solution before CL detection.

The effect of luminol on I/I_0 was investigated at concentrations between 1.0×10^{-6} mol L^{-1} and 5.0×10^{-4} mol L^{-1}. Both signal and background increased with increased luminol concentration. The highest I/I_0 was obtained at 1.0×10^{-5} mol L^{-1} (Figure S1B). As shown in Figure S1C, the effect of laccase concentration on I/I_0 was determined, with I/I_0 rapidly increased with increased laccase concentration, peaking at 0.5 mg mL^{-1}. Further addition of laccase resulted in decreased I/I_0 because of the high background signal occurring under these conditions. Thus, 1.0×10^{-5} mol L^{-1} luminol and 0.5 mg mL^{-1} laccase were chosen for subsequent experiments.

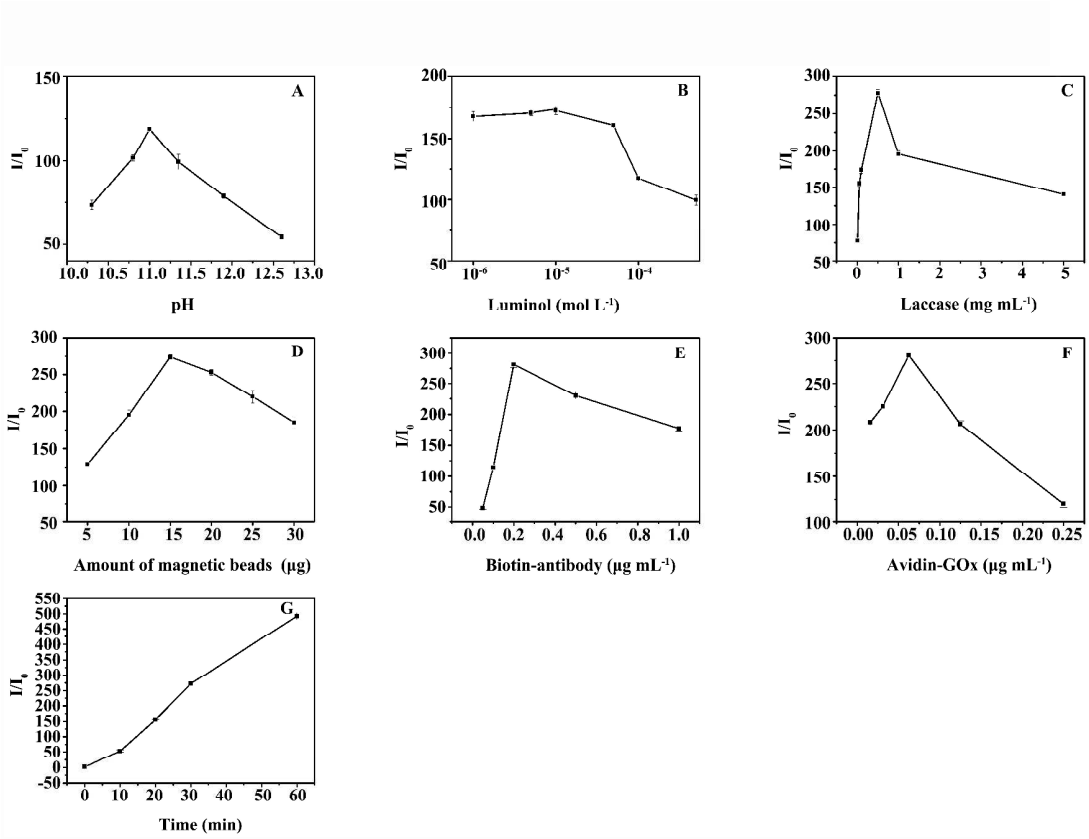

Figure S1. Effects of various conditions on the variance in CL value ratio of I/I_0, where I and I_0 are CL signals with and without *E. coli* O157:H7, respectively. (A) Effect of pH. Conditions: 1.72×10^5 CFU mL^{-1} *E. coli* O157:H7; 0.01 mol L^{-1} glucose; 15.0 μg of IMBs; 1.0×10^{-4} mol L^{-1} luminol; 0.1 mg mL^{-1} laccase; 0.20 μg mL^{-1} biotin–antibody; 0.06 μg mL^{-1} avidin–GOx; and 30 min catalytic time. (B) Effect of luminol concentration. Conditions: pH 11.0; other conditions were the same as in Fig. S1A. (C) Effect of laccase concentration. Conditions: 1.0×10^{-5} mol L^{-1} luminol; pH 11.0; other conditions were the same as in Fig. S1A. (D) Effect of IMB amount. Conditions: pH 11.0; 1.0×10^{-5} mol L^{-1} luminol; 0.5 mg mL^{-1} laccase; other conditions were the same as in Fig. S1A. (E) Effect of biotin–antibody concentration. Conditions: pH 11.0; 1.0×10^{-5} mol L^{-1} luminol; 0.5 mg mL^{-1} laccase; other conditions were the same as in Fig. S1A. (F) Effect of avidin–GOx concentration. Conditions: pH 11.0; 1.0×10^{-5} mol L^{-1} luminol; 0.5 mg mL^{-1} laccase; 0.20 μg mL^{-1} biotin–antibody; other conditions were the same as in Fig. S1A. (H) Effect of catalytic time. Conditions: pH 11.0; 1.0×10^{-5} mol L^{-1} luminol; 0.5 mg mL^{-1} laccase; 0.20 μg mL^{-1} biotin–antibody; 0.06 μg mL^{-1} avidin–GOx; other conditions were the same as in Fig. S1A. Error bars indicate the standard deviations from five independent measurements.

The effect of IMB amount (from 5.0 to 30.0 μg) on I/I_0 was also investigated (Figure S1D). I significantly increased and reached a plateau at an amount of 15.0 μg

of IMBs. Further addition of IMBs did not enhance I, whereas I_0 continually increased under this condition. This phenomenon was probably due to the excess IMBs that induce a slightly non-specific background. As a result, I/I_0 reached its maximumu after the addition of 15.0 μg of IMBs and then decreased. Thus, 15.0 μg of IMBs was added in subsequent experiments.

The effects of the concentrations of biotin-conjugated monoclonal anti-O157 antibody (biotin–antidody) and avidin-conjugated GOx (avidin–GOx) were examined and optimized. First, I/I_0 was observed to increase within the range of 0.05 μg mL^{-1} to 0.20 μg mL^{-1} biotin–antibody and then significantly decrease. Thus, 0.20 μg mL^{-1} of biotin–antidody was selected for subsequent experiments (Figure S1E). Second, the CL and the background signal were increased with the increasing of the avidin-GOx concentration (Figure S1F). The highest I/I_0 was obtained using 0.06 μg mL^{-1} and then decreased. Several GOx samples without avidin coupling and one sample of avidin–GO_X conjugates were respectively incubated with the Anti-O157:H7 IMBs to investigate the role of the GOx and avidin–GOx conjugates for nonspontaneously adsorb onto the beads. After magnetic separation, 100 μL of 0.02 mol L^{-1} glucose in PBS solution (pH 7.4) was then added, and the mixture was incubated with shaking at 37 °C for 30 min. Finally, 100 μL supernatant was mixed with 50.0 μL of laccase and 50.0 μL of luminol, and the CL signals were measured using a multifunctional microplate reader. These CL signals were compared with that of negative control. As shown in Figure S2, the CL signals of samples containing GOx became similar to that of the negative control with increased GOx concentration from 0.01 μg mL^{-1} to 0.50

μg mL^{-1}. However, the CL signal of the sample containing avidin–GOx conjugates was higher than that of the negative control. This finding indicated the avidin–GOx conjugates can cause slightly nonspecific binding because conjugated avidin can nonspecifically bind to certain biological components.[3-5] As a result, I_0 continually increased when the concentration of avidin–GOx conjugates higher than 0.06 μg mL^{-1}, and leading to the decrease of I/I_0. Accordingly, 0.06 μg mL^{-1} of avidin–GOx conjugates was then used in further experiments.

Excess of glucose (0.01 mol L^{-1}) was used for subsequent experiments. The influence of the catalytic time on I/I_0 was also studied. Figure S1G shows that I/I_0 increased at 60 min, which was due to the assumption that the amount of H_2O_2 increased with prolonged catalytic time. Based on this finding and for the sake of rapid analysis, a catalytic time of 30 min was selected for subsequent experiments.

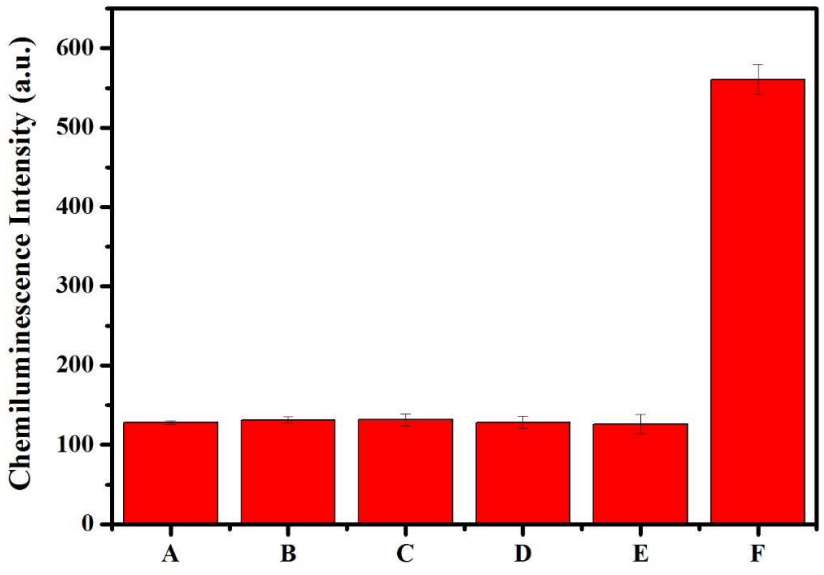

Figure S2. Effect of GOx and avidin–GOx conjugates for nonspontaneously adsorb onto the beads: (A) negative control (without added GOx or avidin–GOx conjugates),

(B) 0.01μg mL^{-1} GOx, (C) 0.05 μg mL^{-1} GOx, (D) 0.1 μg mL^{-1} GOx, (E) 0.5 μg mL^{-1} GOx, and (F) 0.0625 μg mL^{-1} of a avidin–GOx conjugates.

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