Online Supporting Materials

Enzyme catalysis-electrophoresis titration for multiplex enzymatic assay on moving reaction boundary chip

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Peroxidase catalysis oxidation system

Peroxidase catalyzes oxidation of its substrates of luminol in accordance with the following mechanism (**Fig. S1**),[21]

$$E + H_2O_2 \rightarrow EI + H_2O \quad \text{(SI)}$$

$$EI + L \rightarrow EII + L\bullet \quad \text{(SII)}$$

$$EII + L \rightarrow E + L\bullet + H_2O \quad \text{(SIII)}$$

where, E, EI, and EII indicate peroxidase, peroxidase intermediate compounds I and II, respectively, A and L• are luminol and its radical product of one electron oxidation, respectively. The emission wavelength was observed at around 425 nm, which indicated that the luminophore of the luminol- POD-H_2O_2 CL system was the excited-state 3-aminophthalate anion.

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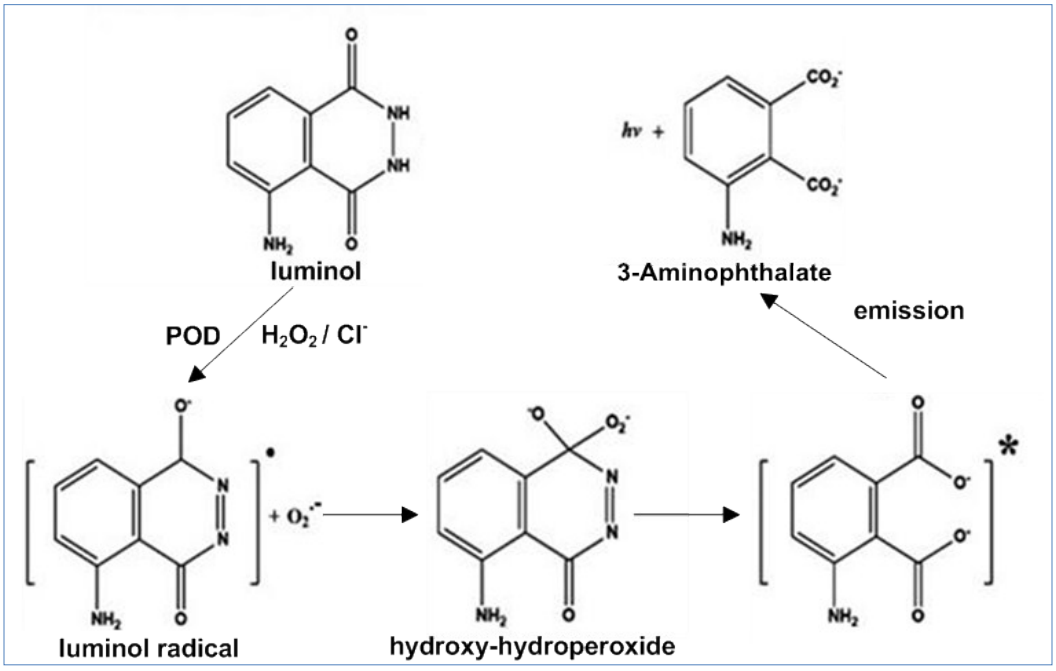

Fig. S1. Chemical reaction of luminol in the system of peroxidase catalyzes oxidation.

Experimental Results

Table S1. Assays of c_{L*}, $c_{L,cu}$, enzyme activity and specific activity via the method of EC-ET chip in **Fig. 4A**and **S3A.***

Enzyme	c (ng/mL)	**2.0**	**4.0**	**6.0**	**8.0**	**20**
HRP	L (mm)	1.1	1.9	3.0	4.2	9.5
	v_{MRB} (m/s)	0.11	0.19	0.3	0.42	0.95
	c_L (mM)	0.13	0.15	0.18	0.24	0.80
	10^{-3} U/mL	1.41	1.62	2.02	2.69	8.84
Laccase	L (mm)	0.70	1.4	2.0	3.0	6.0
	v_{MRB} (m/s)	0.07	0.14	0.2	0.3	0.6
	c_L (mM)	0.14	0.13	0.15	0.18	0.46
	10^{-3} U/mL	1.33	1.49	1.65	3.33	5.12

* The experimental conditions were given in **Fig. 4** and **S3A.**

Table S2. Determination of c_{L*}, $c_{L,cu}$, enzyme activity, specific activity and reaction rate via the method EC-ET chip in **Fig. 4C** and **S3C**.

Enzyme	S (mM)	L (mm)	v_{MRB} (mm/s)	c_{L*} (m)	c_L (mM)	v_{rate} (μM/s)
HRP	0.02	5.78	0.39	9.88	0.12	1.23
	0.05	7.83	0.52	9.82	0.19	1.85
	0.15	9.03	0.60	9.74	0.26	2.34
	0.3	9.25	0.62	9.72	0.28	2.79
	0.5	9.40	0.63	9.71	0.29	2.94
Laccase	0.02	1.62	0.11	9.92	0.07	0.70
	0.05	5.09	0.34	9.89	0.11	1.10
	0.15	7.35	0.49	9.83	0.17	1.66
	0.3	7.84	0.52	9.81	0.19	1.86
	0.5	7.98	0.53	9.81	0.19	1.92

* The experimental conditions were given in **Fig. 4C** and **S3C**.

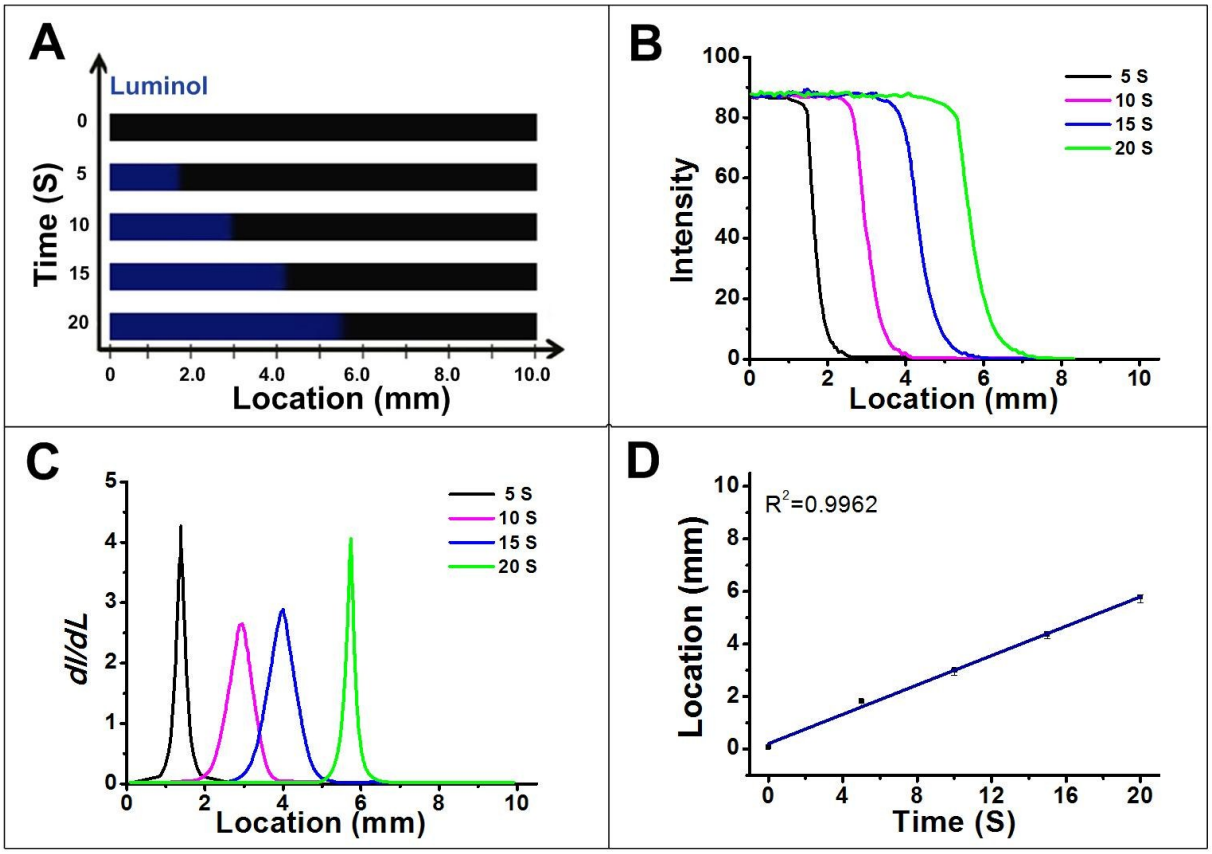

Fig. S2. (A) Boundary motion during the 20 *s* run of EC-ET created with 100 mM Tris-HCl (pH 6.0) in the gel-

filled channel and excited state luminol catalyzed via 6.0×10^{-6} mg/mL laccase in the cathode reservoir. **(B)** Raw intensity *vs* location of MRB obtained by line scanning along the channel axis in **Panel A**. **(C)** Derivatives of sigmoidal curves (*dI/dL vs* location of MRB) transformed from the raw data in Panel B. (**D**) Calibration curve of boundary motion in **Panel C** *vs* the running time of MRB. The other conditions are the same as those in **Fig. 2**. Three measurements were made for each point, the error bars are added to Panel D.

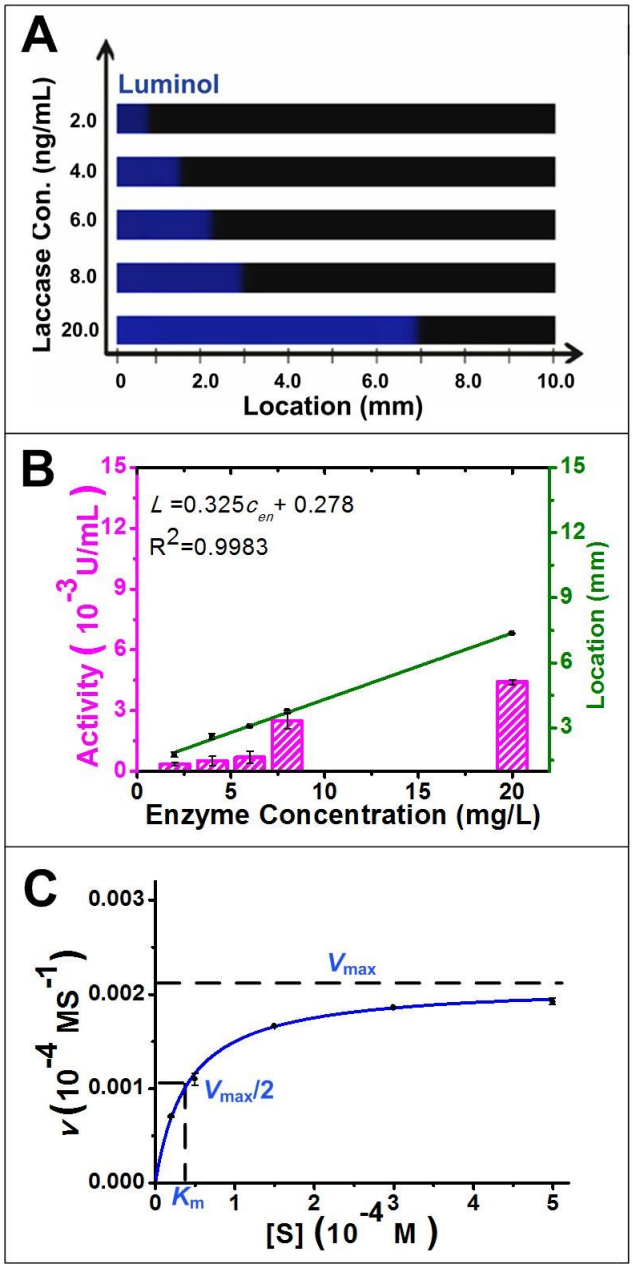

Fig. S3. Multiplex enzyme assay via collateral channel EC-ET chip at different laccase contents and substrate. **(A)** Boundary motion at the 10 *s* run of EC-ET created with 10 mM PBS (pH 6.0) and[3-AP]$^-$ catalyzed via 0, 1.0×10^{-6}, 2.0×10^{-6}, 4.0×10^{-6}, 8.0×10^{-6} and 20 $\times10^{-6}$ mg/mL laccase added into the cathode reservoir; **(B)**

calibration curve of boundary motion vs enzyme content from Panel (**A**): $L = 0.325c_{en} + 0.278$ (R^2=0.9983); **(C)** fitting curve of Michaelis-Menten equation of laccase obtained via collateral channel run of EC-ET at different substrate concentrations of 0.02, 0.05, 0.15, 0.30 and 0.50 mM. The other conditions are the same as those in **Fig. 2**. Three measurements were made for each point, the error bars are added to PanelB, and C.

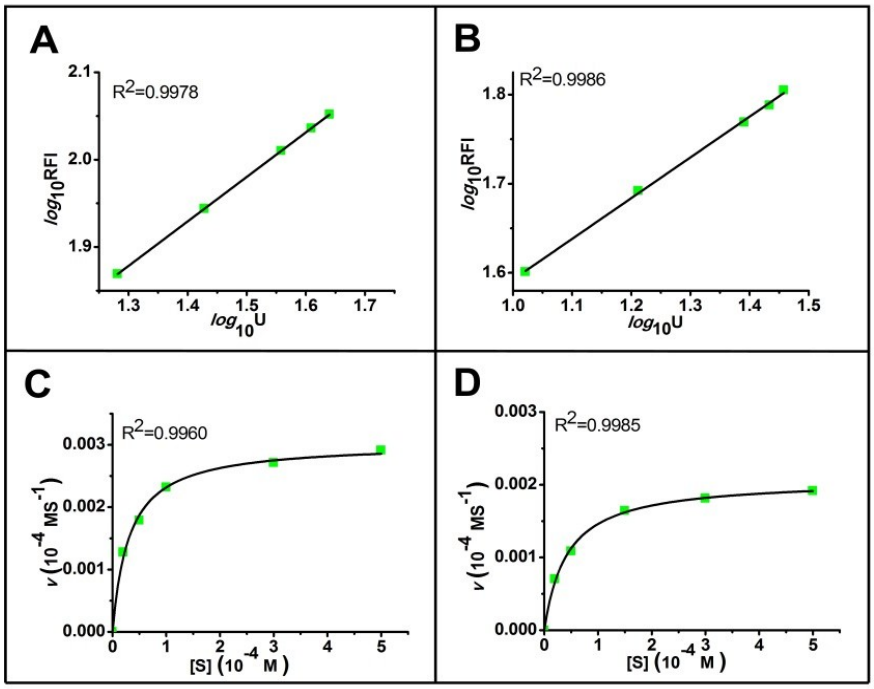

Fig. S4. Fluorescence microplate reader enzyme assay of HRP (**A**) and laccase (**B**), and their relevant Michaelis-Menten plots of HRP (**C**) and laccase (**D**).

Table S3. Relationship of relative fluorescence intensity (RFI) and enzyme activity (U/mL) in the met hod of fluorescence microplate reader of **Fig. S4.***

Sample	RFI *vs* U
HRP	log_{10}RFI = 0.541log_{10}U + 1.17 (R^2=0.9978)
Laccase	log_{10}RFI = 0.457log_{10}U + 1.14 (R^2=0.9986)

* The experimental conditions were given in **Fig. S4**.

Table S4. Assays of enzyme activity and reaction rate via the method of fluorescence microplate reader in **Fig. S4.***

Sample	S (mM)	RFI	U/mL	v_{rate}(μM/s)
HRP	0.02	74.0	19.1	1.28
	0.05	87.9	26.8	1.79

	0.15	102	36.2	2.42
	0.30	109	40.7	2.71
	0.50	113	43.7	2.91
Laccase	0.02	40.4	10.5	0.70
	0.05	48.9	16.3	1.09
	0.15	58.7	24.6	1.64
	0.30	61.2	27.2	1.81
	0.50	62.8	28.7	1.92

* The experimental conditions were given in **Fig. S4**.

Optimization of conditions

pH value. It was shown that the value of chemiluminescence was detected when the catalysis reaction proceeded under the conditions from pH 7.4 to pH 8.8 (**Fig. S5**). The maximum value was obtained at pH 8.3, which was used for the next experiments of EC-ET.

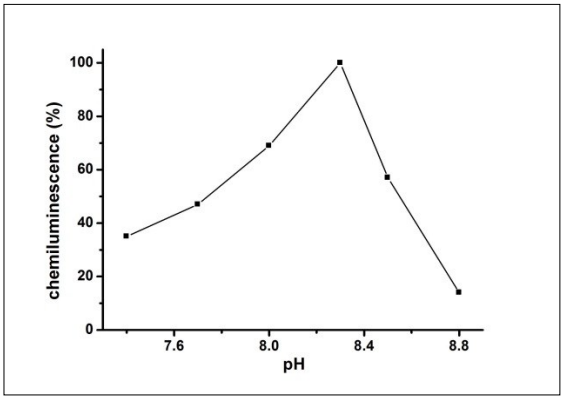

Fig. S5. Effect of pH value of 10 mM Tris-HCl buffer on chemiluminescence intensity produced through HRP-catalyzed oxidation of luminol. Conditions: 2.5×10^{-6} mg/mL HRP, 10 mM luminol, 8.0 mM H_2O_2 and 3.0 mM PIP in 100 mM pH 7.4-pH 8.8 Tris-HCl buffer; room temperature; chemiluminescence intensity recorded after 30 min of catalysis reaction.

Luminol concentration. It was found (**Fig. S6**) that (i) the chemiluminescent signal increased with luminol concentration from zero to 5.0 mM; (ii) the signal reached a saturation if luminol concentration altered from 5.0 mM to 10 mM; and (iii) when luminol concentration was higher than 10 mM, the intensity gradually decreased. On the basis of the obtained results, luminol was set at 10 mM in the following work.

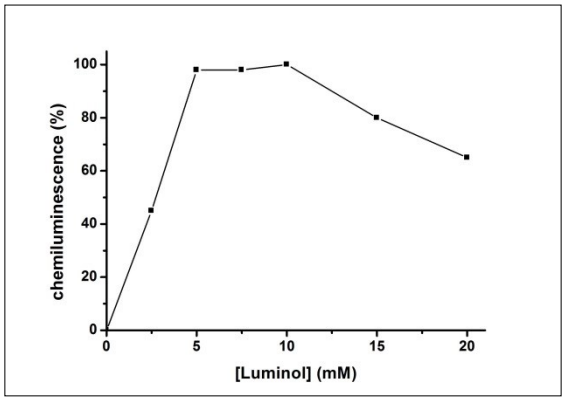

Fig. S6. Effect of luminol concentration on chemiluminescence intensity produced through HRP-catalyzed oxidation of luminol. Conditions: [HRP] = 2.5×10^{-6} mg/mL HRP; 8.0 mM H_2O_2 and 3.0 mM PIP in 100 mM pH 8.3 Tris-HCl buffer; 25°C and chemiluminescence intensity recorded after 30 min of catalysis reaction. The other conditions were the same as those in **Fig. S5**.

H_2O_2 concentration. The maximum chemiluminescent signal was observed at the 8.0 mM of H_2O_2 concentration (**Fig. S7**). A further increase in the concentration of this substrate attenuated the light intensity.

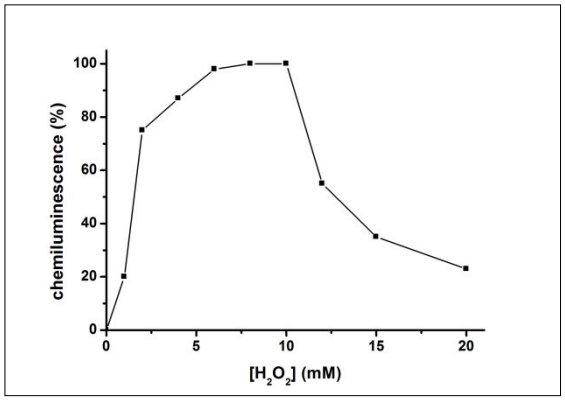

Fig. S7. Effect of H_2O_2 concentration on chemiluminescence intensity produced through HRP-catalyzed oxidation of luminol. The other conditions are the same as those in **Fig. S5**.

PIP concentration.Fig. S9 indicated that the optimized PIP concentration for maximum chemiluminescent intensity was at the concentration of 3.0 mM, and the more and the less PIP content resulted in the decrease of blue light intensity.

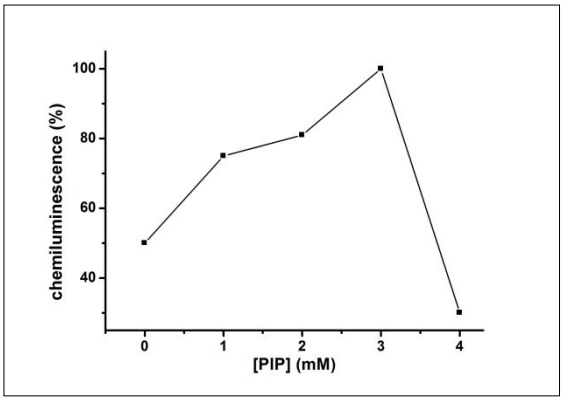

Fig. S8. Effect of PIP concentration on chemiluminescence intensity produced through HRP oxidation of luminol. The other conditions are the same as those in **Fig. S5**.

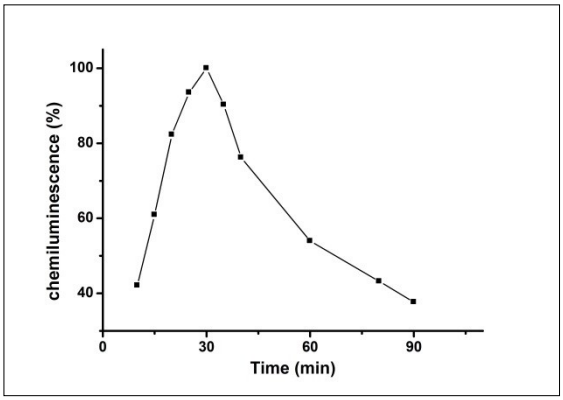

Fig. S9. Effect of reaction time on chemiluminescence intensity produced through HRP oxidation of luminol by hydrogen peroxide. The other conditions are the same as those in **Fig. S5.**

R**eaction time.** The maximum intensity was observed at the 30 minutes of catalysis reaction (**Fig. S9**). The more and the less enzyme reaction time led to the reducing of blue light intensity.

From the optimization of conditions, one gets the following optimized conditions: 10 mM luminol; 2.5×10^{-6} mg/mL HRP, 8.0 mM H_2O_2, and 3.0 mM PIP in 100 mM pH 8.3 Tris-HCl buffer for catalysis reaction; 25 ^{o}C room temperature; and chemiluminescence intensity recorded after 30 min of catalysis reaction. We further conducted the optimization of EC-ET conditions: 12 V/mm electric field; and 10 mM pH 6.0 Tris-HClbuffer in the channel and the anodic reservoir.