Rapid Screening of Hydrogen Bonding Strength from Radicals by Electrochemiluminescence Probes

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Experimental section

Chemicals and materials. Formamide, ethanol, Na_2HPO_4, NaH_2PO_4, NaOH, KOH, H_2SO_4, acetic acid, DMSO, acrylamide and urea were purchased from Beijing Chemical Reagent Company (Beijing, China). Acetamide and methylurea were obtained from Beijing HWRK Chem. Co., Ltd. (Beijing, China). Propionic acid was obtained from Tianjin Fuchen Chemical Reagent Co., Ltd. (Tianjin, China). Butyric acid and trifluoroacetamide were supplied by Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China). *p*-Benzoquinone (*p*-BQ) was offered by Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Nitro blue tetrazolium chloride (NBT) was provided by Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Luminol was obtained from J&K Scientific Ltd. (Beijing, China). The solution of luminol (0.01 M) was prepared by dissolving luminol in 0.1 M NaOH solution. The 0.1 M phosphate buffer solution (PBS, pH=7.5) was obtained by mixing the stock solutions of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. In this work, all reagents were analytical grade and used without further purification. All solutions throughout the experiments were performed under such conditions with Milli Q ultrapure water (18.2 MΩ cm, Millipore, Barnstead, CA, USA).

Apparatus. The cyclic voltammetry electrochemical measurements were conducted with a CHI 660E electro-chemical analyzer (CHI, U.S.A.). A BPCL luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) was used to record the ECL sig-nals. The voltage of the photomultiplier tube (PMT) was set at -900 V (Fig. S1, ESI†). A series of filters (400, 425, 440, 460, 490, 535, 555, 575, and 620 nm) were used to collect the ECL spectra of various samples under CV conditions. The UV-Vis absorption spectra were acquired by a Shi-madzu UV-3600 spectrometer (Tokyo, Japan). The pH values of the samples were measured by a FE-20 pH meter (Shanghai, China). All measurements were conducted at room temperature.

ECL Measurements. Firstly, the gold electrode was polished with 0.30 μM and 0.05 μM alumina slurry successively. Then it was washed with deionized water thoroughly. Subsequently, such gold electrode was sonicated in ethanol for 5 min and then sonicated in deionized water for another 5 min. Next, the cleaned electrode was rinsed electrochemically in 0.5 M H_2SO_4 solution under the voltage from -0.2 to 1.5 V until a stable redox wave of H_2SO_4 was observed. Finally, the gold electrode was washed again by deionized water. In this work, all electrochemical experiments were performed with a conventional three-electrode system. A gold electrode (3 mm diameter) served as the working electrode, a platinum electrode acted as the counter electrode and a saturated Ag/AgCl electrode was the reference electrode. The ECL reactions occurred in 0.1 M PBS (pH = 7.5) containing 0.1 mM luminol in the presence of 1% amides or 1% carboxylic acids. The pH value of each solution was measured by a pH meter, and then adjusted the pH of acid solutions to about 7.5 with 0.1 M NaOH solution. The potential range was set from −0.5 to 0.6 V at the scan rate of 100 mV/s.

Computational details. Quantum chemical calcula-tions were used to obtain the geometries of all monomers and complexes. All of optimized structures and frequency analyses were calculated at the unrestricted B3LYP level using the hybrid DFT functional implemented in the Gaussina09 program package. 6-311++G (2df, 2p) basis set was used for all atoms. Binding energy (BE) has been calculated by subtracting the total energies of the constituent species ‘A’ and ‘B’ from the energy of the complex ‘AB’, as BE(AB) = E(AB) – {E(A) + E(B)}. The thermodynamics (kJ/mol) for association process of $RCONH_2 + HO_2 \rightarrow RCONH_2 \cdots HO_2$ (R=H, CH_3, NH_2, $NHCH_3$) were calculated at B3LYP/6-311++G (2df, 2p) level.

Supporting Figures

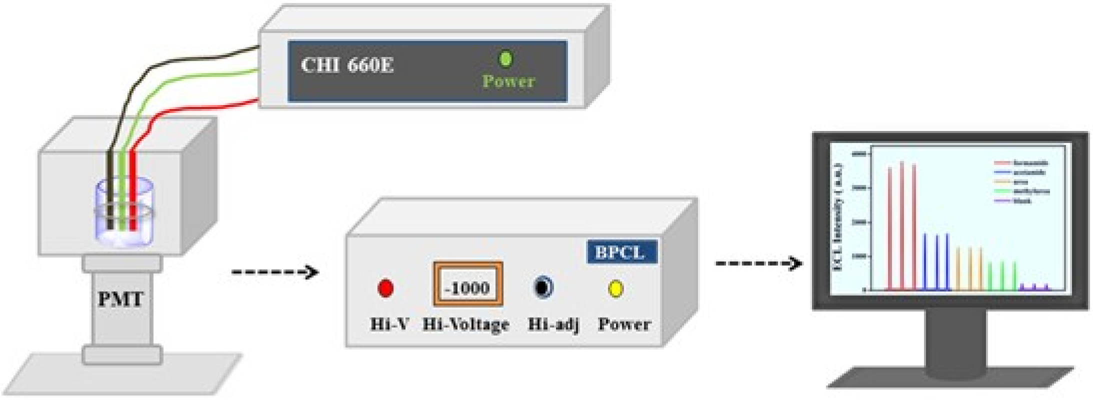


Fig. S1 Schematic diagram of ECL setup.

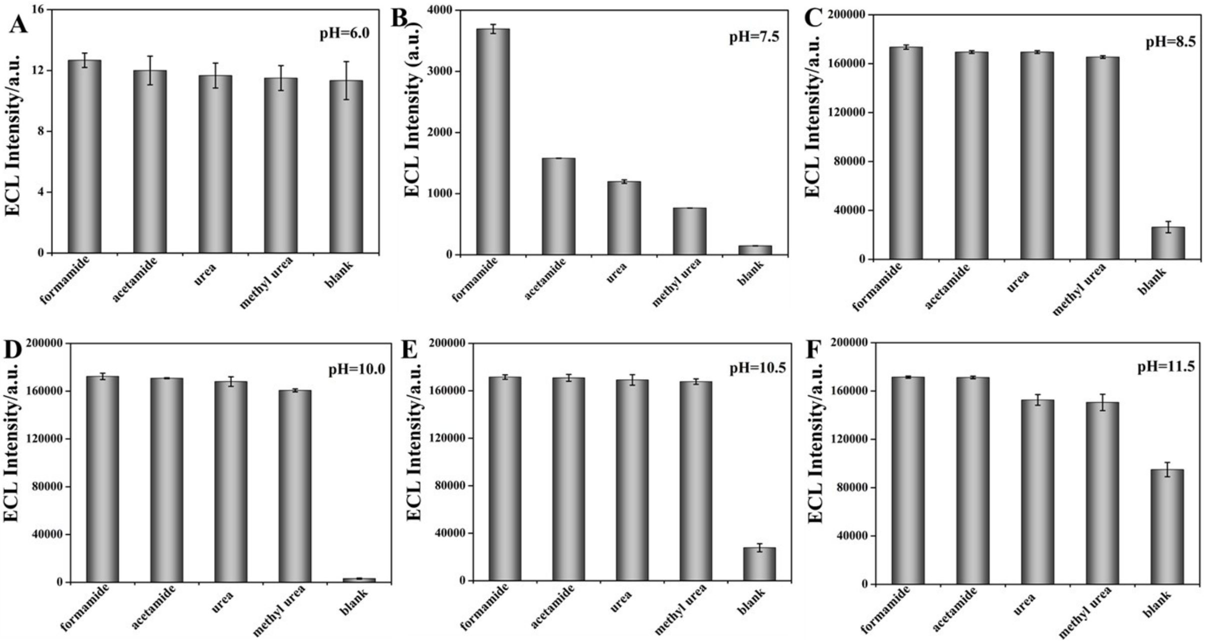


Fig. S2 Optimization of the pH values of the PBS containing 1% amides with 0.1mM luminol on the gold electrode.

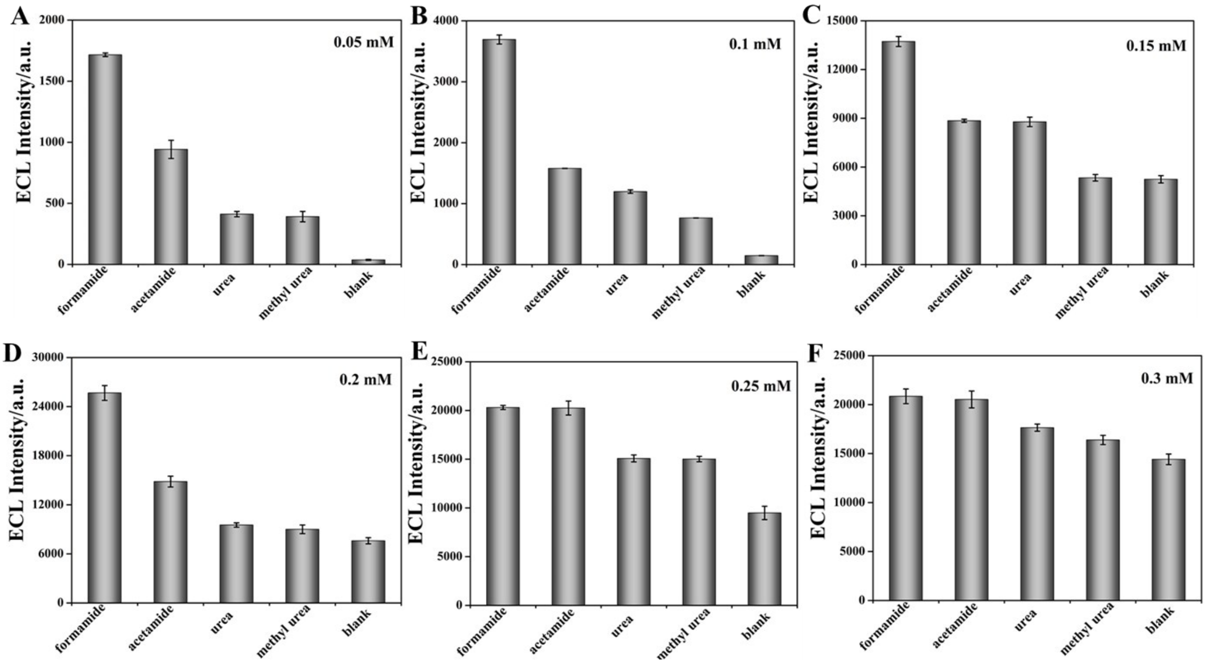


Fig. S3 Optimization of the luminol concentrations in the PBS (pH = 7.5) containing 1% amides on the gold electrode.

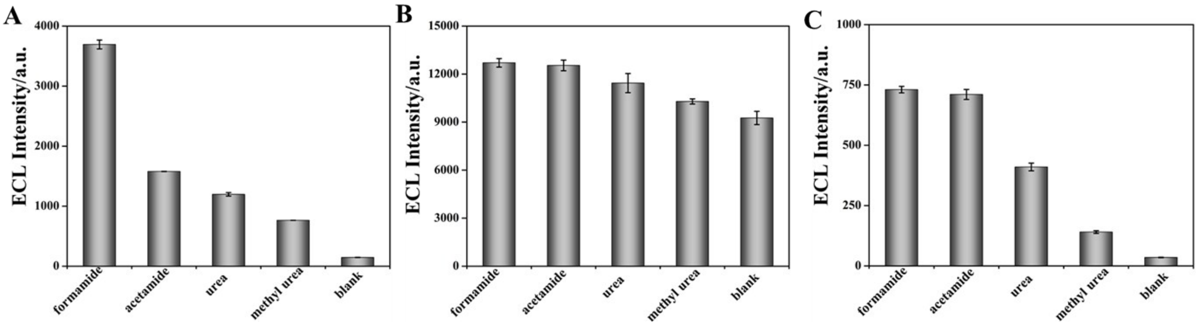


Fig. S4 Effects of the working electrode materials (A) gold electrode; (B) GC electrode; and (C) Pt electrode on the ECL intensity in the PBS (pH = 7.5) containing 0.1mM luminol in the presence of 1% amides.

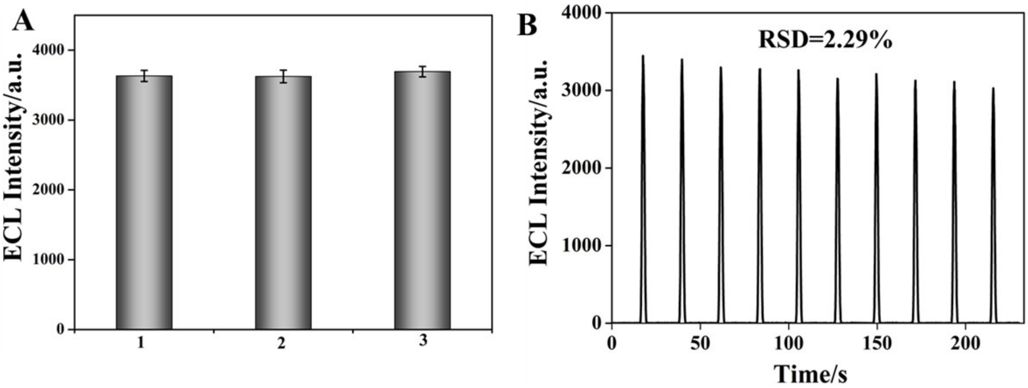


Fig. S5 (A) Repeatability of the ECL system with three different gold electrodes of 0.1 mM luminol in 0.1 M PBS (pH = 7.5) in the presence of 1% formamide in the voltage range from -0.5 to 0.6 V at scan rate of 100 mV/s; (B) Stability of the ECL system for 10 cycles of 0.1 mM luminol in 0.1 M PBS (pH = 7.5) in the presence of 1% formamide on the gold electrode in the voltage range from -0.5 to 0.6 V at scan rate of 100 mV/s.

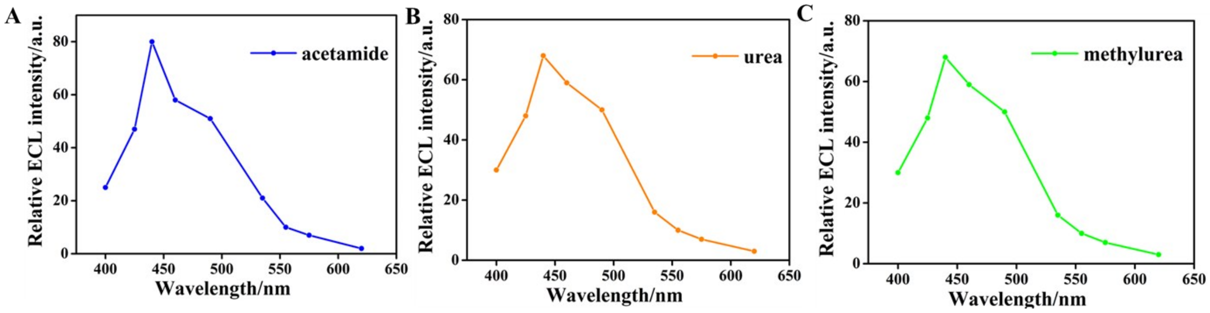


Fig. S6 ECL spectra of luminol in 0.1 M PBS (pH = 7.5) in the presence of (A) 1% acetamide; (B) 1% urea; and (C) 1% methylurea. Scan rate: 100 mV/s.

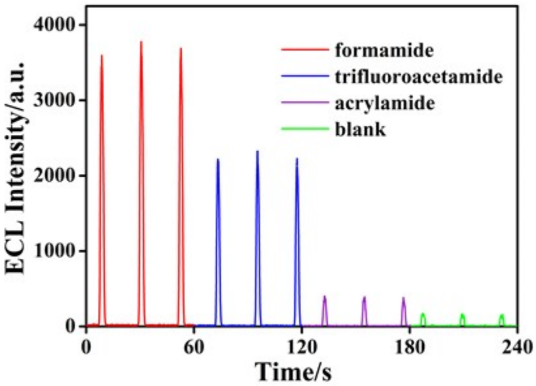


Fig. S7 ECL intensity of 0.1 mM luminol in 0.1 M PBS (pH = 7.5) in the presence of 1% formamide, 1% trifluoroacetamide, and 1% acrylamide in the voltage range from -0.5 to 0.6 V at scan rate of 100 mV/s.

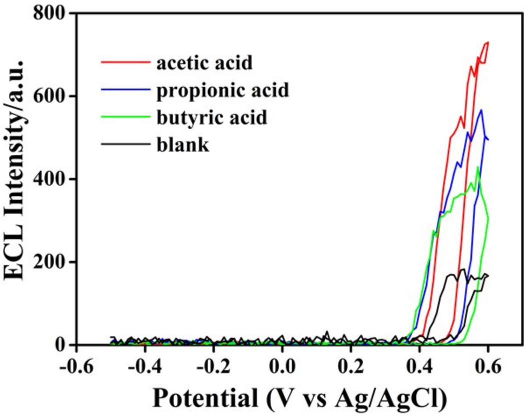


Fig. S8 Cyclic ECL curves of 0.1 mM luminol in 0.1 M PBS (pH = 7.5) in the presence of 1% acetic acid, 1% propionic acid and 1% butyric acid in the voltage range from -0.5 to 0.6 V at scan rate of 100 mV/s.

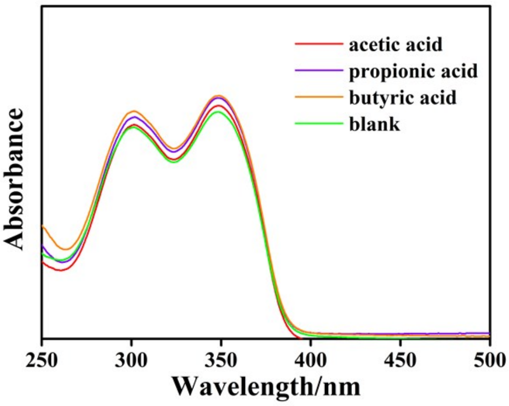


Fig. S9 UV spectra of 1% acetic acid, 1% propionic acid and 1% butyric acid in the presence of 0.1 M PBS solution (pH = 7.5) with 0.1 mM luminol.

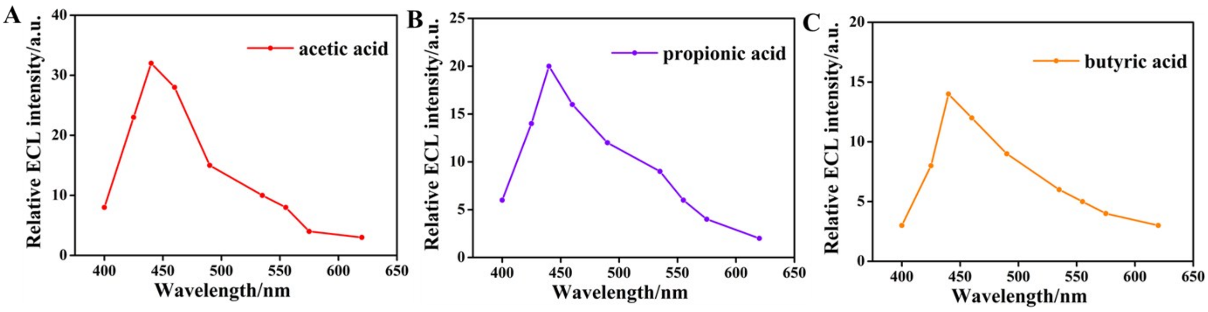
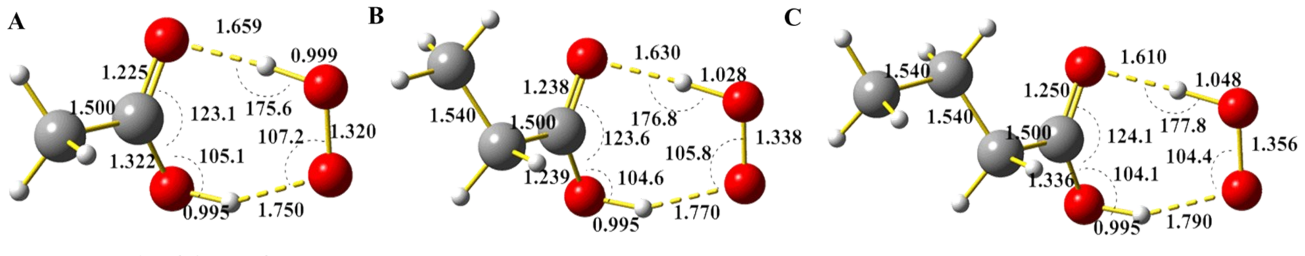


Fig. S10 ECL spectra of luminol in 0.1 M PBS (pH = 7.5) in the presence of (A) 1% acetic acid; (B) 1% propionic acid; and (C) 1% butyric acid. Scan rate: 100 mV/s.



15.46 kcal/mol

15.48 kcal/mol

15.50 kcal/mol

Fig. S11 Most stable geometries of RCOOH···HOO$^{\bullet}$ and BE (kcal/mol) for association process of RCOOH + HO_2→RCOOH···HO_2 (R=CH_3, CH_2CH_3, $CH_2CH_2CH_3$) using B3LYP-6-311++G(2df, 2p) method.

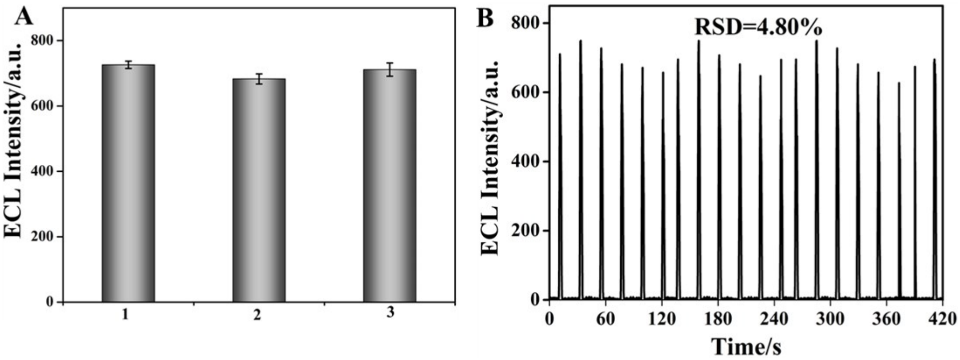


Fig. S12 (A) Repeatability of the ECL system with three different gold electrodes of 0.1 mM luminol in 0.1 M PBS (pH = 7.5) in the presence of 1% acetic acid in the voltage range from -0.5 to 0.6 V at scan rate of 100 mV/s; (B) Stability of the ECL system for 20 cycles of 0.1 mM luminol in 0.1 M PBS (pH = 7.5) in the presence of 1% acetic acid on the gold electrode in the voltage range from -0.5 to 0.6 V at scan rate of 100 mV/s.

Table S1. Thermodynamic parameters for association process of amides with HOO$^\bullet$ radicals at B3LYP-6-311++G (2df, 2p) level.

Association	B3LYP-6-311++G(2df, 2p)		
	ΔE (kJ/mol)	ΔG (kJ/mol)	BE (kcal/mol)
$HCONH_2 + HO_2 \rightarrow HCONH_2 \ldots HO_2$	-39.41	-26.89	9.43
$CH_3CONH_2 + HO_2 \rightarrow CH_3CONH_2 \ldots HO_2$	-40.38	-27.53	9.66
$NH_2CONH_2 + HO_2 \rightarrow NH_2CONH_2 \ldots HO_2$	-40.44	-28.63	9.68
$CH_3NHCONH_2 + HO_2 \rightarrow CH_3NHCONH_2 \ldots HO_2$	-41.60	-29.78	9.95

Table S2. Thermodynamic parameters for association process of carboxylic acids with HOO$^{\bullet}$ radicals at B3LYP-6-311++G (2df, 2p) level.

Association	B3LYP-6-311++G(2df, 2p)		
	ΔE (kJ/mol)	ΔG (kJ/mol)	BE (kcal/mol)
$CH_3COOH+HO_2 \rightarrow CH_3COOH\ldots HO_2$	-64.70	-12.50	15.46
$CH_3CH_2COOH + HO_2 \rightarrow CH_3CH_2COOH\ldots HO_2$	-64.80	-12.59	15.48
$CH_3CH_2CH_2COOH + HO_2 \rightarrow CH_3CH_2CH_2COOH\ldots HO_2$	-64.87	-13.72	15.50

Table S3. Comparison of other reported methods for the screening of hydrogen bonds.

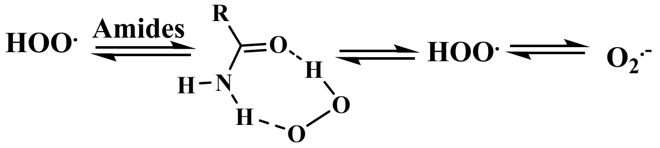
Methods	Targets	Comments	Reference
IR	Hydrogen bonding in 2-Bromohydroquinone	Wide range of application, can characterize the hydrogen bonds between stable compounds, but cannot characterize hydrogen bonds from radicals.	S1
NMR	N–H···N hydrogen bonds		S2
Raman	Intermolecular H-bond in propan-2-ol		S3
DFT calculations	H-bonds between the hydroperoxyl radical and organic acids	Wide application, but time-consuming and sophisticated process.	S4
ECL probe	H-bonds strength between the hydroperoxyl radical and amides/carboxylic acids	Rapid screening of the hydrogen bonding strength from radicals, quick response, low cost, good stability and easy operation.	Our work

$$LH^{-} - e^{-} \longrightarrow LH^{\cdot} \longrightarrow L^{\cdot -} + H^{+} \qquad (1)$$

$$L^{\cdot -} + O_2 \longrightarrow O_2^{\cdot -} + L \qquad (2)$$

$$O_2 + H_2O + 2e^{-} \longrightarrow HOO^{-} + OH^{-} \qquad (3)$$

$$HOO^{-} - e^{-} \longrightarrow HOO^{\cdot} \rightleftharpoons O_2^{\cdot -} \qquad (4)$$



(5)

$$L^{\cdot -} + O_2^{\cdot -} \longrightarrow LO_2^{2-} \qquad (6)$$

$$LO_2^{2-} \longrightarrow AP^{2-*} + N_2 \qquad (7)$$

$$AP^{2-*} \longrightarrow AP^{2-} + hv \qquad (8)$$

Scheme S1. ECL mechanism of luminol in the proposed system.

References

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[S2] A. J. Dingley, L. Nisius, F. Cordier and S. Grzesiek, *Nat. Protoc.*, 2008, **3,** 242–248.

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