Electronic Supplementary Information (EIS)

Electrochemiluminescent enantioselective detection with chiral-imprinted mesoporous metal surfaces

Sopon Butcha,^{‡a,b} Jing Yu,^{‡a} Zikkawas Pasom,^{a,b} Bertrand Goudeau,^a Chularat Wattanakit,^{*b} Neso Sojic,^{*a} and Alexander Kuhn^{*a,b}

Abstract: Chiral-imprinted mesoporous Pt-Ir alloy surfaces were successfully combined in a synergetic way with electrochemiluminescence (ECL) to detect the two enantiomers of phenylalanine (PA) as a model compound, acting simultaneously as a chiral target and also as a co-reactant to generate significant differences in ECL signals. The chiral features encoded in the metal surfaces are converted into an enantioselective electrogeneration of the excited state of the [Ru(bpy)3]2+ dye, which in fine produces the differentiating light emission with up to 20-fold differences in intensity for the two enantiomers. These findings open up the possibility of developing ECL-based bioassays with molecular-imprinted metals and microscopy of chiral environments.

DOI: 10.1039/x0xx00000x

Table of Contents

Contents	Pages
Experimental procedures	3
Chemicals	3
Synthesis of chiral-encoded mesoporous Pt-Ir alloy electrodes	3
Characterization of chiral-encoded mesoporous Pt-Ir alloy electrodes	3
Electrochemistry and electrochemiluminescence (ECL) analysis	3
Results and Discussion	4
Fig. S1 Optimized structures of phenylalanine enantiomers	4
Fig. S2 Surface characterization by SEM of the imprinted Pt-Ir alloy	4
Fig. S3 Electrochemical and ECL behavior of the analyte	5
Fig. S4 Reproducibility experiments	5
Fig. S5 Influence of scan rate	6
Fig. S6 Estimation of detection limit	6
Fig. S7 Stability test	7
Scheme S1 Proposed mechanism of ECL generation	7
References	7
Author Contributions	7

Experimental Procedures

Chemicals

L-phenylalanine (L-PA) was purchased from Tokyo Chemical Industry (TCI), and D-phenylalanine (D-PA), chloroplatinic acid hydrate (H₂PtCl₆·xH₂O), dihydrogen hexachloroiridate (IV) hydrate (H₂IrCl₆·xH₂O), polyethylene glycol hexadecyl ether (Brij[®]C10), sodium phosphate dibasic heptahydrate (Na₂HPO₄·7H₂O), sodium phosphate monobasic monohydrate (NaH₂PO₄·H₂O), Tris(2,2'-bipyridyl) dichlororuthenium (II) hexahydrate (Ru(bpy)₃Cl₂·6H₂O), and 2-propanol were received from Sigma-Aldrich. 1 M of Phosphate buffer solution (PBS) with a solution pH of 7.33 was prepared from Na₂HPO₄·7H₂O and NaH₂PO₄·H₂O dissolved in MilliQ water. Sulfuric acid (H₂SO₄) was obtained from Alfa. MilliQ water (18.2 MΩ·cm at 25 °C) was employed for all experiments. All chemicals were used without further purification.

Synthesis of chiral-encoded mesoporous Pt-Ir alloy electrodes

The synthesis of the electrodes was carried out by electrochemical deposition with a three-electrode system using Ag/AgCl (sat. KCl), a Pt mesh (geometric area of 1 cm²), and the prepared Pt-Ir alloy electrodes (geometric area of 0.25 cm²) as a reference, counter and working electrodes, respectively, connected to a potentiostat (Metrohm μ Autolab Type III). All recordings were analyzed using the Nova (version 1.11) software.

Gold-coated glass slides were cleaned by sonicating in 2-propanol for 15 min and then rinsing with MilliQ water several times before drying under nitrogen (N₂) gas flow. The chiral-imprinted mesoporous Pt-Ir electrodes were prepared following a previously reported procedure with some modifications,¹ using L- or D-phenylalanine (L- or D-PA) as the different chiral templates. The alloy electrodes have been obtained by co-electrodeposition from a gel containing 28 wt% of Pt and Ir salts (H₂PtCl₆·6H₂O and H₂IrCl₆·6H₂O, with 85 at% Pt and 15 at% Ir, respectively) in the presence of non-ionic surfactants (Brij® C10, 28 wt%), forming in hexagonal lyotropic liquid crystal phase, dissolved in MilliQ water (36 wt%), and the chiral templates, either L-PA or D-PA with a molar ratio of 1 PA / 12 (PtCl₆²⁻ + IrCl₆²⁻). All electrodes were generated on cleaned gold-coated glass slides as support by applying a potential of -0.05 V vs. Ag/AgCl. The thickness of the deposited layer was controlled by choosing a deposition charge density of 4 C cm⁻². Finally, the templates, including chiral molecules and non-ionic surfactants, were entirely removed by rinsing several times and immersing the electrodes in MilliQ water overnight.

Characterization of chiral-encoded mesoporous Pt-Ir alloy electrodes

Scanning Electron Microscopy (SEM) was used to explore the surface morphology of the Pt-Ir electrodes using a Hitachi TM-1000 tabletop microscope. Cross-sectional imaging of the electrodes was performed by SEM on a JEOL, JSM-7610F. The mesoporous structure of the Pt-Ir alloys was confirmed by Transmission Electron Microscopy (TEM) carried out on a JEOL JEM-ARM200F microscope at 200 kV. To prepare a Pt-Ir layer for TEM measurements, the deposited Pt-Ir layers were removed from the substrate by gentle mechanical scraping. The samples were dispersed in ethanol and transferred to copper (Cu)-TEM grids for analysis. To verify the electrochemically active surface area (ECSA), cyclic voltammetry (CV) was used by scanning the mesoporous Pt-Ir electrodes in $0.5 \text{ M H}_2\text{SO}_4$ at a scan rate of 100 mV s⁻¹. The ECSA of the prepared electrodes was calculated from the hydrogen adsorption/desorption regions of the obtained CV curves following the literature.²

Electrochemistry and electrochemiluminescence (ECL) analysis

The chiral discrimination was studied by differential pulse voltammetry (DPV). All experiments were carried out with a step potential of 10 mV, a modulation amplitude of 50 mV, a modulation time of 50 ms, and an interval time of 500 ms in a potential window ranging from -0.1 to 1.3 V. Only the control experiment with a slower scan rate (Figure S5) was performed with a step potential of 5mV instead of 10mV. Cyclic voltammetry (CV) was performed with a scan rate of 100 mV s⁻¹ in a potential window ranging from -0.1 to 1.5 V. Both techniques were employed for characterizing solutions with various concentrations of PA and [Ru(bpy)₃]²⁺ dissolved in 0.1 M PBS solution as supporting electrolyte (pH 7.33). The analysis was carried out either with a classical Pt disk electrode (1.6 mm diameter) or with the chiral-imprinted Pt-Ir electrodes as working electrodes, using a platinum mesh (geometric area of 1 cm²) as a counter electrode, and Ag/AgCl/KCl (sat. KCl) as reference electrode, connected to a μ Autolab type III potentiostat. Prior to analysis, the Pt disk electrode was polished with 0.25 μ m diamond paste (Struers) and rinsed with ethanol and MilliQ water several times. Regeneration of the electrode surfaces was achieved by chronoamperometry at -0.1 V for 30 s in 0.1 M PBS solution.

ECL signals were collected in a homemade cell with a Hamamatsu R5070 photomultiplier tube (PMT detector) placed at a distance of approximately three millimeters in front of the working electrode. The PMT detector was held at -750 V with a Hamamatsu C9525 high-voltage power supply. The obtained signal was amplified by a Keithley 6485 Picoammeter, and the resulting signal was acquired *via* a second input channel of the μ Autolab type III potentiostat. Prior to analysis, all electrodes were soaked in the solution containing only 10 mM L- or D-PA dissolved in 0.1 M PBS solutions for an hour. After that, the working electrodes were placed in the solution containing 10 mM L-PA or D-PA/1 mM [Ru(bpy)₃]²⁺ dissolved in 0.1 M PBS solutions, and the ECL signals were immediately recorded. The experiments were carried out in a dark room.

Enantioselectivity between L- and D-phenylalanine (PA) was calculated following relationship (1) depending on the PA target enantiomer:

with I_L and I_D being the maximum ECL intensities, which were measured in L-PA and D-PA solutions, respectively.



Fig. S1 Optimized structures of phenylalanine (PA) enantiomers.

The chemical structures were obtained by an optimization method *via* Density Functional Theory (DFT) calculations (6-31G(d,p) B3LYP) using the Gaussian program (09W).



Fig. S2 Top-view of the SEM image of the chiral imprinted mesoporous Pt-Ir electrode. The Pt-Ir alloy is generated by electrodeposition with a deposited charge density of 4 C cm^{-2} .



Fig. S3 Control experiments demonstrating the behavior of mesoporous Pt-Ir alloys in different solutions and using different techniques. (a) Cyclic voltammetry displays a significant capacitive current combined with the ECL signal (CV-ECL); (b) Effect of PA concentration on ECL intensity using CV-ECL; (c) ECL intensity as a function of the composition of the solution recorded by DPV-ECL. In this study, luminophore and co-reactant have to be present simultaneously to generate a significant ECL signal. In the case of CV-ECL, the analyte solutions contain different concentrations of L-PA, ranging from 10, 20 to 30 mM, and 10 μ M [Ru(bpy)₃]²⁺ dissolved in 0.1 M PBS solution (pH 7.33), analyzed with a scan rate of 100 mV s⁻¹. The activated mesoporous Pt-Ir alloys were immersed in the solution 1 h before analysis. DPV-ECL experiments were performed in a solution containing 10 mM L-PA and 1 mM [Ru(bpy)₃]²⁺ dissolved in 0.1 M PBS solution (pH 7.33). As described in the experimental section, chronoamperometric surface regeneration was applied before each analysis.



Fig. S4 ECL intensity recorded with a,b,c) three different L-PA imprinted Pt-Ir electrodes and d,e,f) three different D-PA imprinted Pt-Ir electrodes in a solution composed of either 10 mM L-PA and 1 mM $[Ru(bpy)_3]^{2+}$, or 10 mM D-PA and 1 mM $[Ru(bpy)_3]^{2+}$, dissolved in 0.1 M PBS solution (pH 7.33)). The average ratio of enantioselectivity for all these imprinted electrodes without activation is 3.15±0.96, indicating clearly a preferential reaction of the imprinted enantiomer.



Fig. S5 Normalized ECL intensity recorded with an L-PA imprinted Pt-Ir electrode in two solutions composed of 10 mM L-PA and 1 mM $[Ru(bpy)_3]^{2+}$, 10 mM D-PA and 1 mM $[Ru(bpy)_3]^{2+}$ dissolved in 0.1 M PBS solution (pH 7.33), respectively. The DPV was performed with a step potential of 5 mV, a modulation amplitude of 50 mV, a modulation time of 50 ms, and an interval time of 500 ms.



Fig. S6 DPV and ECL intensity recorded with a commercial Pt electrode in a solution composed of a) 0.01, b) 0.1, c) 1mM D-PA and 1 mM $[Ru(bpy)_3]^{2+}$ dissolved in 0.1 M PBS solution (pH 7.33) in the same light sensitivity range. DPV was performed with a step potential of 10 mV, a modulation amplitude of 50 mV, a modulation time of 50 ms, and an interval time of 500 ms.



Fig. S7 ECL signals from stability test of a D-PA imprinted Pt-Ir alloy for the discrimination of PA enantiomers. (a) - (e) Normalized ECL curves recorded with the two amino acid enantiomers in five consecutive cycles. The experiments were conducted by scanning in both 10 mM L-PA and D-PA/1 mM $[Ru(bpy)_3]^{2+}$ dissolved in 0.1 M PBS solutions, using a D-PA imprinted mesoporous Pt-Ir electrode and the DPV-ECL technique. Potential induced surface regeneration was performed before each analysis. The ECL signals recorded for the fifth cycle demonstrate a loss of enantioselectivity within error bars (0.8 ± 0.3). DPV was performed with a step potential of 10 mV, a modulation amplitude of 50 mV, a modulation time of 50 ms, and an interval time of 500 ms.

$$PA \rightarrow PA^{+\bullet} + e^{-}$$
 (Eq. 1)

$$PA^{+} \rightarrow PA^{+} + H^{+}$$
 (Eq. 2)

$$Ru(bpy)_{3}^{2+} \rightarrow Ru(bpy)_{3}^{3+} + e^{-}$$
 (Eq. 3)

$$Ru(bpy)_{3^{3^{+}}} + PA^{\bullet} \rightarrow Ru(bpy)_{3^{2^{+^{*}}}} + PA$$
(Eq. 4)

$$\operatorname{Ru}(\operatorname{bpy})_{3}^{2^{+*}} \rightarrow \operatorname{Ru}(\operatorname{bpy})_{3}^{2^{+}} + hv \tag{Eq. 5}$$

Scheme S1 Proposed mechanism of ECL generation in the present case. In this present study, we propose an ECL pathway, which is similar to the one described in a previous report for the well-known $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ system.³ Briefly, the reaction route is mainly based on three steps: (i) and (ii) describing the direct oxidation of PA (Eq. 1 and 2) and $\text{Ru}(\text{bpy})_3^{2+}$ (Eq. 3) at the Pt-Ir electrode surface, respectively, and (iii) the $\text{Ru}(\text{bpy})_3^{3+}$ reduction induced by the PA• radical, generating the excited state (Eq. 4) which leads to light emission (Eq. 5).

References

- 1 S. Butcha, S. Assavapanumat, S. Ittisanronnachai, V. Lapeyre, C. Wattanakit, A. Kuhn, Nat. Commun., 2021, 12, 1314.
- 2 (a) J. M. Doña Rodríguez, J. A. Herrera Melián, J. Pérez Peña, J. Chem. Educ., 2000, 77, 1195; (b) M. Łukaszewski, M. Soszko, A. Czerwiński, Int. J. Electrochem. Sci., 2016, 11, 4442-4469.
- 3 (a) W. Miao, J.-P. Choi, A. J. Bard, J. Am. Chem. Soc., 2002, 124, 14478-14485; (b) M. M. Richter, Chem. Rev., 2004, 104, 3003-3036.

Author Contributions

S. B. and Y. J. performed with equal contribution all experiments, data collection, and writing of the original draft. Z.P. carried out additional control experiments. B. G. was associated with the experimental ECL set-up and investigation. C. W., N. S., and A. K. provided supervision, funding acquisition, writing and editing of the manuscript.