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Supporting Information

ATP-responsive Laccase@ZIF-90 as a Signal Amplification Strategy Achieving Indirect Highly Sensitive Online Detection of ATP in Rat Brain

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EXPERIMENTAL SECTION

Materials and Reagents. $Zn(CH_3COO)_2 \cdot 2H_2O$, imidazole-2-carboxaldehyde (2-ICA), laccase, adenosine triphosphate (ATP), dopamine (DA), uric Acid (UA), ascorbic acid (AA), adenosine diphosphate (ADP), adenosine monophosphate (AMP), 3,4-dihydroxyphenylacetic acid (DOPAC) and sodium dodecyl sulfate (SDS) were all purchased from Sigma (Shanghai, China). Other chemicals were purchased from Beijing Chemical Reagent Company (Beijing, China). All reagents were of analytical reagent grade and used as received. The aqueous solutions were prepared with deionized (DI) water produced by a Milli-Q system (Millipore, Bedford, MA, USA, 18.2 MΩ•cm). The artificial cerebrospinal fluid (aCSF) (126 mM NaCl, 2.4 mM KCl, 1.1 mM $CaCl_2$, 0.85 mM $MgCl_2$, 27.5 mM $NaHCO_3$, 0.5 mM Na_2SO_4, 0.5 mM KH_2PO_4, pH 7.4) was used as perfusion solution for on-line measurements.

Preparation and Characterization of Laccase@ZIF-90 Microcrystals. Laccase@ZIF-90 microcrystals were prepared according to the previous method.[1] 9 mg laccase was added to 3 mL 0.04 M 2-ICA aqueous solution under stirring, followed by adding 0.001 M $Zn(CH_3COO)_2 \cdot 2H_2O$ aqueous solution. After 10 min of stirring, the reaction mixture was centrifuged, the resulted Laccase@ZIF-90 were washed twice. The size and morphology of the prepared Laccase@ZIF-90 were characterized using transmission electron microscope (TEM). Further characterizations were performed by X-ray diffraction (XRD), thermal gravity analysis (TGA) and Fourier transform infrared spectroscopy (FT-IR) analysis.

To confirm the protein is encapsulated in ZIF-90 rather than adsorbed on the external surface of ZIF-90, Laccase@ZIF-90 were washed with 0.1 g/mL SDS solution, followed by FT-IR characterization. For comparison, the as-prepare ZIF-90 were mixed with laccase solution, followed by similar SDS washing and FT-IR characterization.

Protein Encapsulated and Released from Laccase@ZIF-90. To determine the protein encapsulation efficiency of Laccase@ZIF-90, the supernatant of preparation mixture was collected and mixed with 5 mM DA, UV-Vis was used to measure the

absorbance value at 480 nm. A certain amount of laccase was added to the supernatant. By making a standard curve, the encapsulation of laccase into Laccase@ZIF-90 was calculated to be 90.3%.

To determine and study the ATP-triggered protein released by Laccase@ZIF-90, Laccase@ZIF-90 were incubated with different concentrations of ATP at the same time, the mixture was then centrifuged, and the supernatant was collected to quantify laccase. The amount of laccase released into the solution was calculated by calibrating the absorbance value at 480 nm by UV-Vis.

Continuous Monitoring of ATP with the OECS. Surgeries for in vivo microdialysis and global cerebral ischemia were performed with the methods reported previously.[2, 3] Briefly, adult male Sprague-Dawley rats (SD rats) (weighing 300 ± 50 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). The rats were housed individually with a 12:12 h light/dark schedule and with food and water available ad libitum. Briefly, the rats inserted with guide tube were anesthetized by chloral hydrate (350 mg/kg i.p.). The microdialysis probe (2 mm in length; Bioanalytical Systems Inc. (BAS), BAS Carnegie Medicine) was implanted into the brain cortex region (AP=0.2 mm, L=5.6 mm from bregma, V=3 mm from the surface of the skull) according to standard stereotaxic procedures. Throughout the surgery, the body temperature of the rats was maintained at 37 °C with a heating pad. After the surgery, the rats were placed into a warm incubator individually until they recovered from anesthesia. The rats could recover for a day before microdialysis and on-line electrochemical detection. The microdialysis sampling was perfused with aCSF at a flow rate of 1 μL/min to monitor cerebral ATP level, and the microdialysates were introduced into OECS for continuous measurements. Then, the surgical procedures for two-vessel occlusion (2-VO) ischemia were undertaken during the period of in vivo microdialysis and on-line electrochemical measurements. Global ischemia model was constructed with occlude of the bilateral common carotid's arteries with nontraumatic arterial clips.[4]

Supporting Figures.

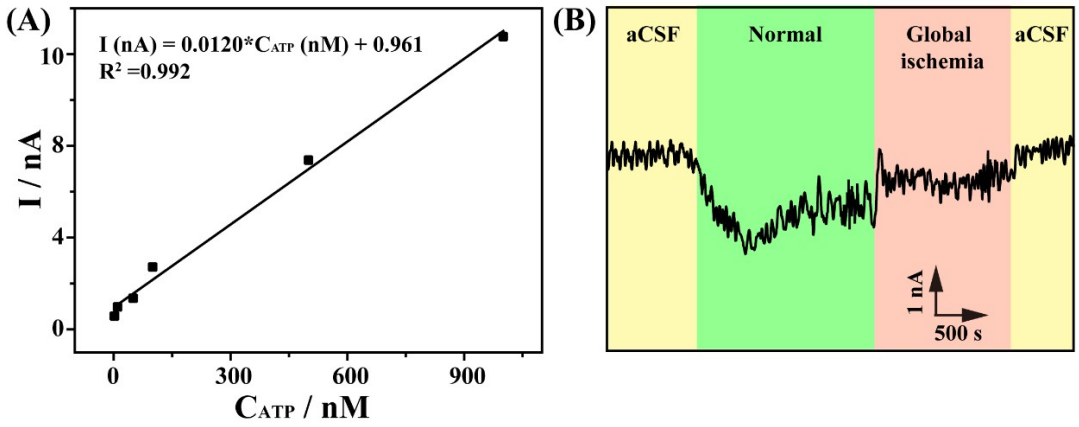

Figure S1. (A) Plot of current response vs. ATP concentration. (B) Continuous monitoring the dynamic changes of ATP level in living SD rat brain before and after 2-VO global cerebral ischemia

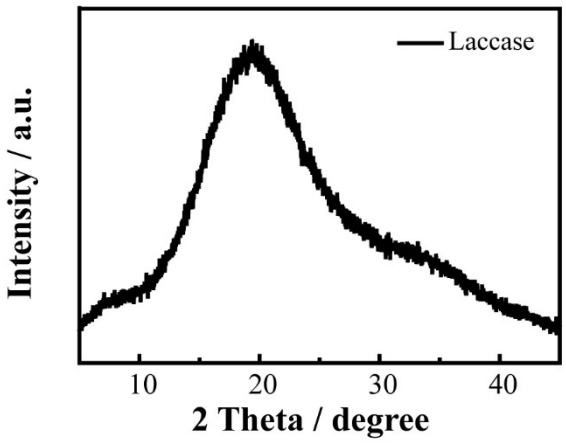

Figure S2. XRD pattern of laccase.

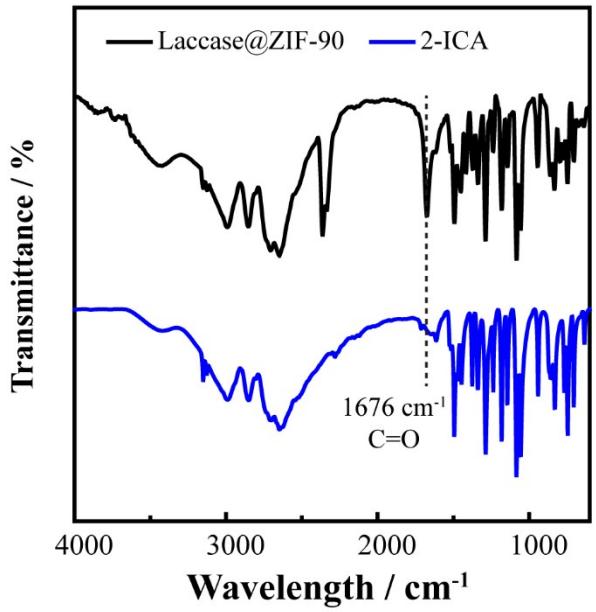

Figure S3. FT-IR spectra of Laccase@ZIF-90 and 2-ICA ligand.

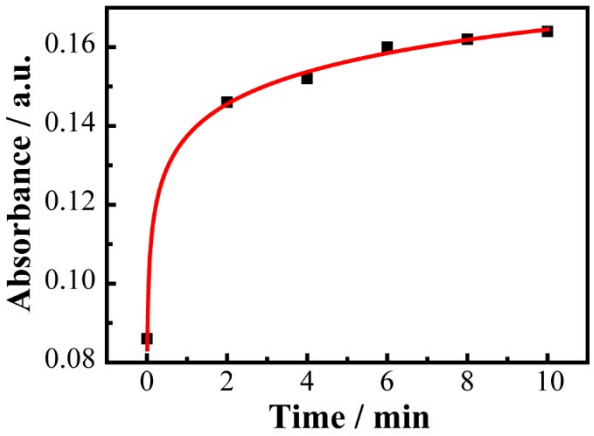

Figure S4. Kinetics curve of laccase release after the ATP destroying Laccase@ZIF-90.

Discussion of TGA and FT-IR.

In Figure 2 D, the first gradual weight-loss step of ~22% up to 80 °C corresponds to the removal of H_2O and guest molecules (e. g., 2-formaldehyde imidazole) along with desorption of some gases from cavities. The second decomposition stage of the composite started from 80 °C and finished around 200 °C, showing about ~8% of weight loss attributed to the decomposition of protein molecules within MOF.[5] The final

weight loss for both pure ZIF-90 and the composite, starting from ~300 to 800 °C of about ~70% corresponds to the decomposition of the ZIF-90 framework.[6]

Figure 3B represents the characteristic peaks of ZIF-90, Laccase@ZIF-90, Laccase@ZIF-90 treated with ATP and laccase. The peak at ~3730 cm^{-1} results from the stretching vibration of N-H in imidazole-2-carboxyaldehyde and the stretching vibration of the C-H in aldehyde group appears at ~2850 cm^{-1}. The peak at ~1676 cm^{-1} is ascribed to the stretching vibration of the C=O in aldehyde group and the peak at 1455 cm^{-1} represents N-H vibration from C-N stretching, respectively. Peaks at 955 cm^{-1} could be ascribed to the bending vibration of C-H.[6] Peaks at 2933cm^{-1} and 1239 cm^{-1} could be ascribed to the bending vibration of -COOH, while those at 1646 cm^{-1} and 1081 cm^{-1} are corresponding to amide I (mainly from C=O stretching mode) and the stretching vibration of C-N, respectively.[5]

Table S1. Comparison of Different Sensors for the Detection of ATP.

Sensor	Linear range	Detection limit	Application	Analytical method	Reference
Aptazymes DNAzymes	1-200 nM	0.6 nM	Human serum	Electrochemical	7
DNA-templated CuNPs	0.2-50 μM	93 nM	Cell medium	Fluorescence	8
β-CD-$CuInS_2$ QDs/aptamer	6-1200 μM	3μM	Vitro detect	Fluorescence	9
Aptamer	0.1-1000 μM	33 nM	Human serum and cell extracts	Fluorescence	10

Aptamer DNAzyme	1−50000 0 μM	-	Vitro detect	Fluorescence	11
DNAzyme	5-230 nM	2.4 nM	Human serum	Electrochemical	12
Micrometer scale ion current rectification	5-100 nM	-	Rat brain	Electrochemical	13
Laccase@ZIF -90	2-1000 nM	0.12 nM	Rat brain	Electrochemical	This work

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