Supporting Information

An Implantable Ionic Liquid-Gel Microelectrode for In Vivo Monitoring of K^+ Levels in the Living Rat Brain

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1. Experimental Procedures

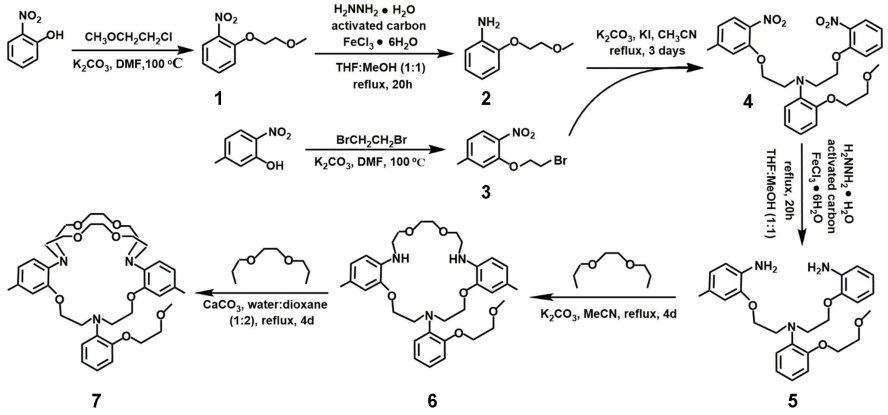
1.1 Chemicals and Reagents. 1-Butyl-3-vinylimidazolium bromide, 2,2-azobis (2-methylpropionitrile), 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (C_2M), 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (C_4M), 1-decyl-3-methylimidazolium bis(Trifluoromethanesulfonyl)imide ($C_{10}M$), lithium bis(trifluorometh-anesulphonyl) imide (LiTFSI) and 1,2-dichloroethane were purchased from Adamas. Ascorbic acid (AA), dopamine (DA), uric acid (UA), cysteamine (Cyst), glucose and 30% H_2O_2 were purchased from Sigma-Aldrich (Shanghai, China). NaCl, KCl, LiCl, $CaCl_2$, $ZnCl_2$, $MgCl_2·6H_2O$, $CuCl_2·2H_2O$, $NiCl_2·6H_2O$, $CoCl_2·6H_2O$, NaOH, $NaNO_3$, Na_2CO_3, $NaHCO_3$, Na_2SO_4, Na_2SO_3, $NaNO_2$ and NaClO were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-phenylalanine (Phe), L-methionine (Met), glycine (Gly), L-glutamine (Glu), L-cysteine(Cys), L-arginine (Arg), L-lysine (Lys), L-leucine (Leu), L-serine (Ser), L-threonine (Thr), L-valine (Val) and D(+)-glucose were bought from Co., Ltd. (Beijing, China). Fluorescein isothiocyanate labelled bovine serum albumin solution (BSA-FITC) was purchased from BoOlsen Biotechnology Co. Ltd (Beijing, China). All aqueous solutions were prepared with deionized water obtained from Milli-Q water (18.2 MΩ cm^{-1}). All chemicals were of analytical grade and were used without further purification. Unless otherwise specified, all experiments were performed at room temperature (ca. 25 ºC).

1.2 Synthesis of PB. Poly(1-butyl-3-vinylimidazolium bis(trifluoro-methylsulfonyl)imide (PB) was synthesized by free radical polymerization in ethanol as follows. 1-Butyl-3-vinylimidazolium bromide (2.31 g, 10.00 mmol), 2,2-azobis (2-methylpropionitrile) (0.016 g, 0.10 mmol) and ethanol (5 mL) were added to a 25 mL round bottom flask, and reacted for 24 h under stirring. Then, after rotating the ethanol, the mixture was poured into an excess of acetone to produce white precipitate. The precipitate was dried and then dissolved in water. The solution (5 mL) of 3.00 g LiTFSI was added dropwise to get the product of PB. After being dried under vacuum at 40 °C, PB was a faint yellow solid with a yield of 90%. ^{1}H-NMR (600MHz, acetone-D6) δ(ppm): 0.83 (3H, $-CH_2–CH_2–CH_3$), 1.29 (2H, $-CH_2–CH_2–CH_3$), 1.75 (2H, $–CH_2–CH_2–CH_3$), 2.54(2H,

–CH_2–CH–N-), 4.05 (1H, –CH_2–CH–N-), 7.22 (1H, –N–CH=CH–N-CH_2-), 7.72 (1H, –N–CH=CH–N-CH_2-), 8.59 (1H, –N=CH–N–).

1.3 Synthesis routes of TAC and C2.

TAC was synthesized as reported previously as follows (Scheme 1):[26-27]



Scheme S1. Synthetic routes of TAC (7) and C2 (6).

Compound 1: A suspension of 140 g (1010 mmol) 2-nitrophenol, 105g (1110 mmol) chloroethyl methyl ether, 84.2 g (507 mmol) KI, 153 g (1110 mmol) K_2CO_3 and 500 mL DMF was heated at 100 °C for 6 h. Solvent was evaporated and the residue was dissolved in 500 mL $CHCl_3$ and 500 mL water. Organic phase was washed with 500 mL 2.5% Na_2CO_3 and 500 mL sat. NaCl, dried over Na_2SO_4. The solvent was evaporated to afford 161 g (81%) light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ=3.45 (s, 3H), 3.78 (t, 2H), 4.25 (t, 2H), 7.02-7.82 (m, 4H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ=151.63, 134.75, 125.29, 121.08, 115.69, 70.51, 69.38, 58.79, 40.48, 40.31, 40.14, 39.98, 39.81, 39.64, 39.48 ppm. HRMS (ESI, m/z): Calcd for $C_9H_{11}NO_4$ ($[M^+H]^+$) 54.82; Found: 54.63.

Compound 2: 60.5 g (30.7mmol) compound 1 was dissolved in 200 mL methanol, 3.0 g 10% palladium on activated carbon was added. This suspension was hydrogenated at 2.2 atm. for 18 h, till no more hydrogen uptake was observed. The catalyst was filtered off and the solvent was evaporated to afford 48.7 g (95%) light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ=3.45 (s, 3H), 3.65 (s, 2H), 3.78 (t, 2H), 4.20 (t, 2H), 6.74-6.82 (m, 4H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ=145.92, 138.54, 121.82, 116.72, 114.58, 113.07, 71.06, 68.16, 58.70, 40.47, 40.31, 40.14, 39.97,

39.81, 39.64, 39.47 ppm. HRMS (ESI, m/z): Calcd for $C_9H_{13}NO_2$ ($[M^+H]^+$): 64.65; Found: 64.27.

Compound 3: 122.5 g (800 mmol) 5-methyl-2-nitrophenol, 751.0 g (4000 mmol) 1,2-dibromoethane, 110.7 g (800 mmol) K_2CO_3 were suspended in 400 mL anhydrous DMF. Heated at 120 °C for 1 h. Cooled, most of the liquid was evaporated. The residue was dissolved in 1 L $CHCl_3$ and 1 L water. The organic layer was washed with 2×1 L 1.8% NaOH till the aqueous layer became pale yellow. The organic layer was dried over Na_2SO_4. Filtered and the solvent was evaporated to give ~240 g oil. Triturate with 240 mL boiling methanol. Sat for 2 h. The resulting precipitate was filtered and washed with 2×100 mL cold methanol, dried at room temperature for 18 h. Afforded 103.4 g (50%) offwhite crystal with a melting point 45-47 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ=2.40 (s, 3H), 3.65 (t, 2H), 4.30 (t, 2H), 6.85-7.75 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ=151.25, 146.26, 137.75, 125.58, 122.08, 116.28, 69.66, 40.16, 39.83, 39.50, 31.13, 21.76 ppm. HRMS (ESI, m/z): Calcd for $C_9H_{10}BrNO_3$ ($[M^+H]^+$): 41.56; Found: 41.96.

Compound 4: A mixture of $CaCO_3$ (2.0 g, 20 mmol) and KI (3.32 g, 20 mmol) in 30 mL distilled water was heated to reflux under argon atmosphere. Then a solution of 1 (7.80 g, 30 mmol) and 2 (1.67 g, 10 mmol) in 20 mL degassed 1,4-dioxane was added. The resulting reaction mixture was stirred under reflux for 16 h. Then more compound 3 (2.60 g, 10 mmol) was added and the reaction mixture was refluxed for another 20 h. After cooling to room temperature, the mixture was filtered and the filtrate was condensed to 20 mL. Then the filtrate was extracted with CH_2Cl_2 (20 mL×3). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by flash column chromatography using CH_2Cl_2 provided 4 as a yellow solid (4.76 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.73 (d, 2H), 7.07 (dd, 1H), 6.98-6.84 (m, 5H), 6.75 (d, 2H), 4.21 (t, 4H), 4.14-4.07 (m, 2H), 3.75 (t, 4H), 3.73-3.67 (m, 2H), 3.35 (s, 3H), 2.36 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ=152.9, 152.7, 145.9, 139.1, 137.5, 125.8, 123.2, 122.1, 121.7, 120.9, 115.2, 114.6, 71.3, 68.3, 68.0, 59.0, 52.4, 22.0 ppm. HRMS (ESI, m/z): calcd for $C_{27}H_{31}N_3O_8$ ($[M^+H]^+$) 526.2111; found 526.2118.

Compound 5: To a solution of 4 (4.73 g, 9.0 mmol) in 25 mL anhydrous THF was added activated carbon (0.27 g) and a pre-dissolved 25 mL methanol solution of $FeCl_3{\cdot}6H_2O$ (0.49 g, 1.8 mmol) under argon atmosphere. The resulting reaction mixture was heated to reflux and then hydrazine

monohydrate (9.0 g, 180 mmol) was added dropwise. After refluxing for 20 h, the reaction mixture was cooled to room temperature and filtered. The residue was washed with 10 mL THF and the combined filtrate was condensed. Then the crude mixture was dissolved into 50 mL CH_2Cl_2 and washed with brine (50 mL×2). The organic layer was dried over $MgSO_4$, filtered and condensed to give the desired compound 5 as a light-yellow liquid (4.03 g, yield 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.09 (d, 1H), 7.00-6.94 (m, 1H), 6.93-6.85 (m, 2H), 6.59-6.50 (m, 6H), 4.18-4.09 (m, 6H), 3.77-3.69 (m, 6H), 3.40 (s, 3H), 2.21 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ=152.8, 146.5, 139.7, 134.2, 127.9, 123.1, 122.2, 121.6, 121.4, 115.2, 113.9, 113.2, 71.3, 67.7, 67.2, 59.1, 52.6, 21.0 ppm. HRMS (ESI, m/z): calcd for $C_{27}H_{35}N_3O_4$ ($[M^+H]^+$) 466.2628; found 466.2632.

Compound 6: A mixture of K_2CO_3 (0.42 g, 3.0 mmol) and 50 mL degassed acetonitrile was heated to reflux under argon atmosphere. Then, a solution of 4 (0.47 g, 1.0 mmol) and 1,2-bis(2-iodoethoxy)ethane (0.41 g, 1.1 mmol) in 50 mL degased acetonitrile was added dropwise over 4 h. The resulting reaction mixture was stirred under reflux and the reaction progress was monitored by LC-MS. 4 days later, the LC-MS results indicated that the reaction completed. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was extracted with CH_2Cl_2/H_2O and washed three times with brine. The organic layer was dried over $MgSO_4$, then filtered and condensed. The crude product was further purified by flash column chromatography using 2.5% methanol/CH_2Cl_2 as eluent to give 6 as an off-white solid (0.23 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.15-7.08 (m, 1H), 6.98-6.86 (m, 3H), 6.68-6.60 (m, 2H), 6.53-6.46 (m, 4H), 4.18-4.10 (m, 2H), 4.03 (t, 4H), 3.85 (t, 4H), 3.79-3.71 (m, 6H), 3.69 (s, 4H), 3.44 (s, 3H), 3.37-3.24 (m, 4H), 2.20 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ=152.4, 146.7, 138.6, 136.0, 126.3, 122.7, 121.8, 121.7, 121.4, 114.4, 112.1, 110.5, 71.3, 70.9, 70.0, 68.0, 67.7, 59.2, 53.4, 44.1, 21.0 ppm. HRMS (ESI, m/z): calcd for $C_{33}H_{45}N_3O_6$ ($[M^+H]^+$) 580.3308; found 580.3295.

TAC: A mixture of $CaCO_3$ (0.30 g, 3.0 mmol) and 50 mL distilled water was heated to reflux under argon atmosphere followed by the addition of 50 mL deoxygenated 1,4-dioxane. Then a solution of 6 (0.58 g, 1.0 mmol) and 1, 2-bis(2-iodoethoxy)ethane (0.37 g, 1.0 mmol) in 50 mL

deoxygenated 1, 4-dioxane was added dropwise over 4 h. The resulting reaction mixture was stirred under reflux and the reaction progress was monitored by liquid chromatography mass spectrometry (LC-MS). The LC-MS results indicated that the reaction was completed after four days. After cooling to room temperature, the mixture was filtered and the filtrate was condensed to 50 mL. Then, the filtrate was extracted with CH_2Cl_2 (50 mL×3). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by flash column chromatography using 3.0% methanol/CH_2Cl_2 provided TAC as a colorless foamy solid (0.48 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.13 (dd, 1H), 6.97-6.85 (m, 5H), 6.64 (d, 2H), 6.56 (d, 2H), 4.23-4.16 (m, 2H), 3.99-3.86 (m, 8H), 3.81-3.78 (m, 2H), 3.76-3.57 (m, 16H), 3.48-3.27 (m, 11H), 2.22 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ=153.4, 152.4, 138.5, 138.2, 132.7, 122.7, 121.8, 121.6, 121.0, 114.4, 114.03, 71.36, 71.3, 70.2, 68.0, 67.3, 59.2, 54.0, 53.2, 21.2 ppm. HRMS (ESI, m/z): calcd for $C_{39}H_{55}N_3O_8$ ($[M^+H]^+$) 694.3989; found 694.3978.

1.4 Preparation of the ILG-TAC electrode. Using P-2000 capillary laser Instrument (Sutter Instrument) to draw micron sized quartz microtube probe, the hole diameter is in micron size. In order to form a stable interface, the prepared micropipets were filled with ILG-TAC composed of ILG (1/1, wt/wt) and 200 mM TAC. The micropipette exhibited a round orifice with a radius of ~2.5 μm. ILG microelectrodes were fabricated through injecting the hot gel solution from the back of the micropipette using a small syringe. After cooling down to the room temperature, the position of L/L interface was examined using microscopy to ensure that no air bubbles were inside the micropipette, and the micropipette had a nice contact with the gel phase.

1.5 *In Vivo* Experiments. All animal experiments were performed according to the guidelines of the Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of China and were approved by the Animal Care and Use Committee of East China Normal University (Shanghai, China). The experimental and surgical methods were made according to our previous reports with modification. Adult male Wistar rats with a weight of 150-200 g were chosen as experimental animals. The rats were anesthetized with gas isoflurane (ABS small

animal gas anesthesia machine, Shanghai Yuyan Scientific Instrument Co., Ltd), in which the induction and anesthesia concentration of isoflurane was 3.5%, and the continuous anesthesia concentration was 2.5%. Then, the rat was fixed on a stereotaxic frame (Beijing Tide - Gene Biotechnology Development Centre) and the ILG-TAC electrode was implanted in the cortex (L=5.6 mm, AP=0.2 mm from bregma, V=2.0 mm below dura). The reference and counter electrodes were placed in a 2 mm plastic cannula and then implanted in the dura of the brain. During the surgery, the rat was wrapped in a heating pad to maintain body temperature at 37 °C and the ILG-TAC electrode was implanted slowly and carefully within 30 min to reduce the damage.

Results and Discussion

2. ^{1}H NMR and ^{13}C NMR of TAC and C2.

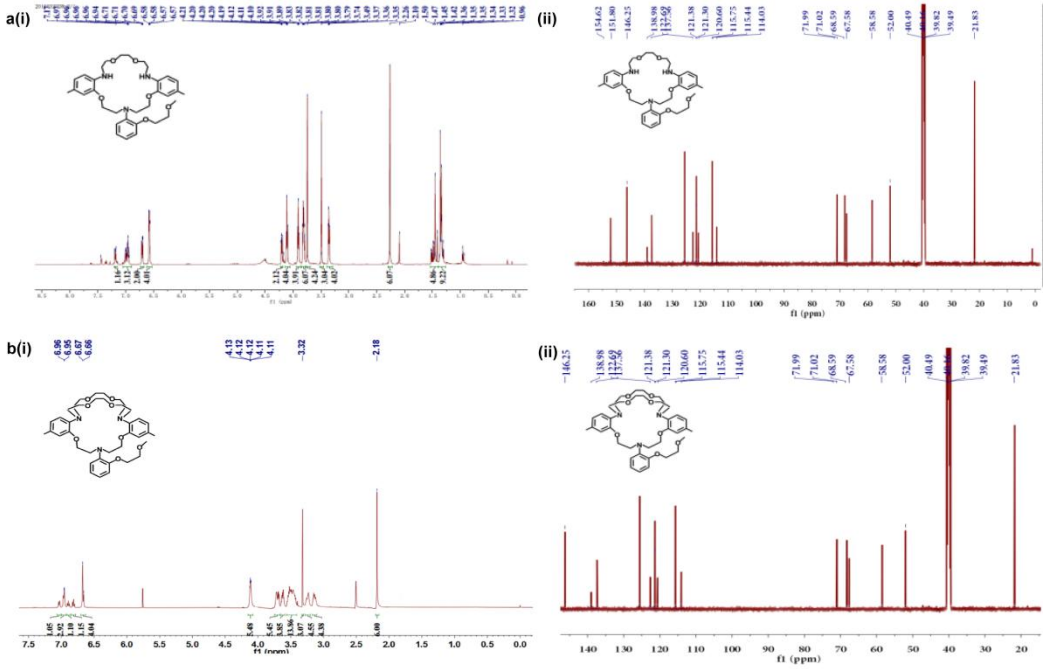


Figure S1. ^{1}H NMR spectra (i) and ^{13}C NMR spectra (ii) of C2 (a) and TAC (b) in $CDCl_3$.

3. FT-IR and ^{1}H NMR of PB .

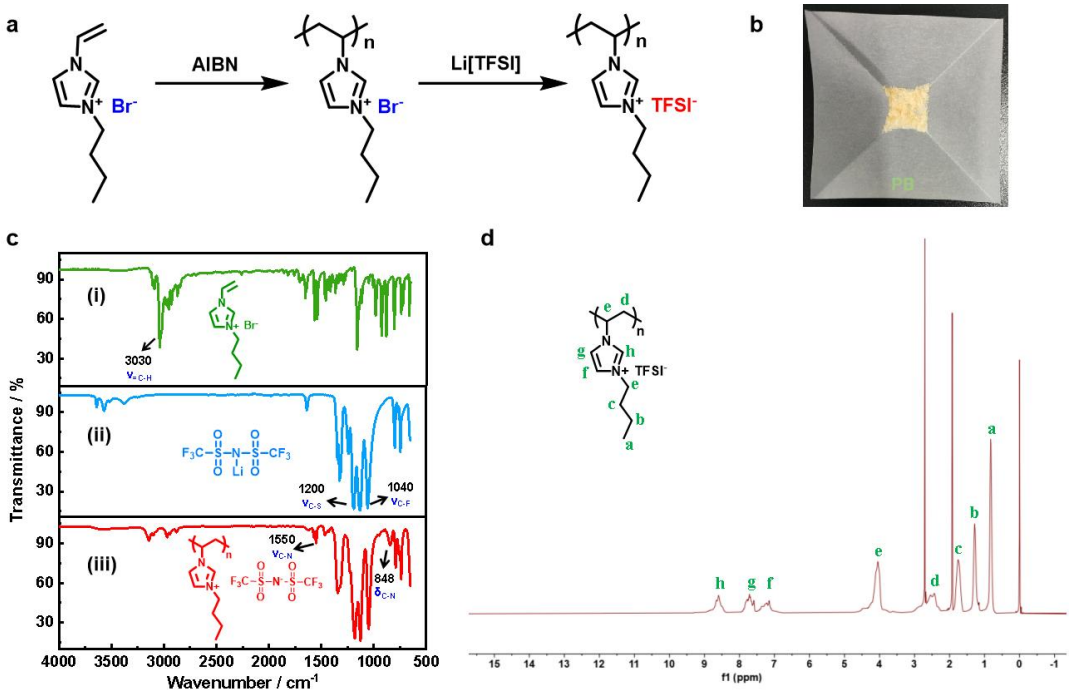


Figure S2. (a) Synthetic routes of PB. (b) Photograph of PB. (c) FT-IR spectrum of (i) 1-butyl-3-vinylimidazolium bromide, (ii) lithium bis(trifluoromethylsulfonyl)imide and (iii) PB. (d) ^{1}H NMR spectrum of PB.

4. Polarized potential windows of different ionic liquids.

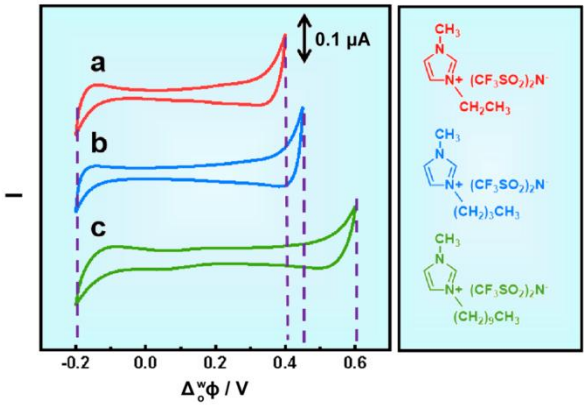


Figure S3. Typical CVs at (a) C_2M/W, (b) C_4M/W, and (c) C_{10}M/W interfaces. Scan rate: 10 mV s^{-1}.

5. The position of ILG-TAC/W interface

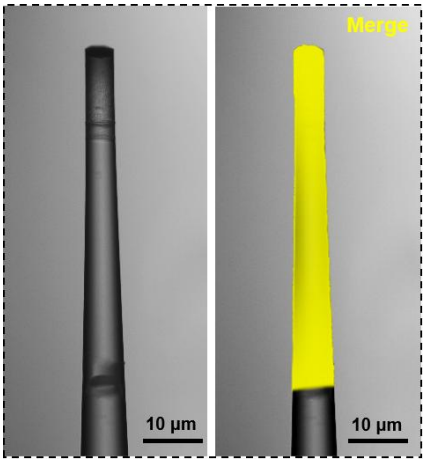


Figure S4. Confocal image (left) and fluorescence image (right) of the micropipette filled with fluorescein-labeled ILG-TAC.

6. The effect of micropipette silanization on the electrochemical performance.

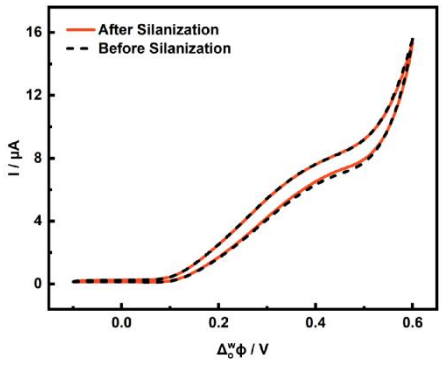


Figure S5. CV curves obtained at ILG-TAC microelectrode before and after silanization.

7. Response time.

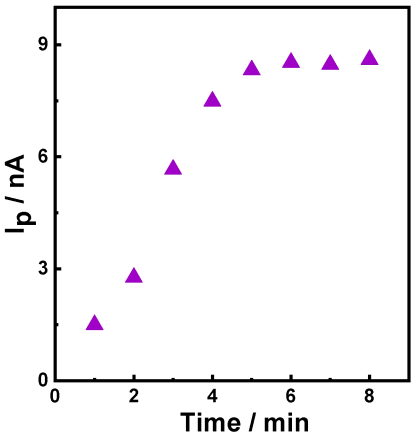


Figure S6. The relationship between I_p and reaction time obtained at the ILG-TAC electrode containing 5 mM K^+.

8. Optimized mass ratio of $C_{10}M$ to PB.

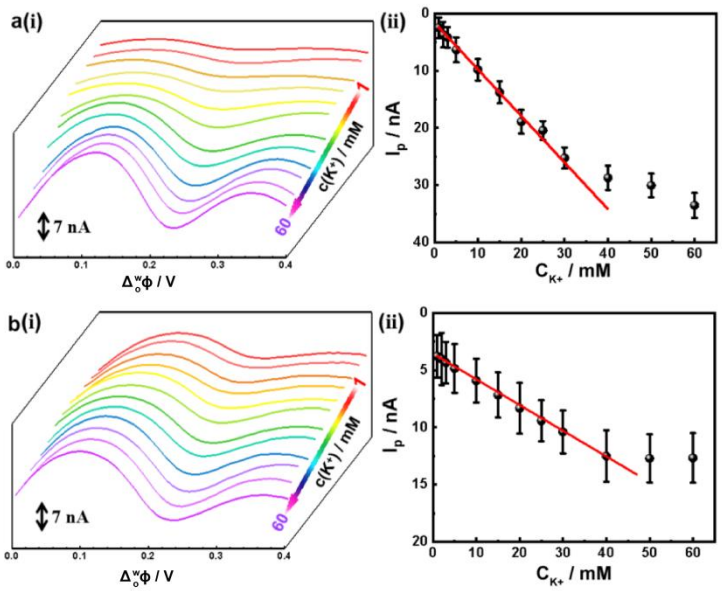


Figure S7. DPV curves of ILG-TAC electrodes in response to different K^+ concentrations under (a) 2:1 and (b) 1:2 mass ratio of $C_{10}M$ to PB (i), and the corresponding calibration curve (ii).

9. Electrochemical performance of the PVC-TAC gel electrode toward K^+.

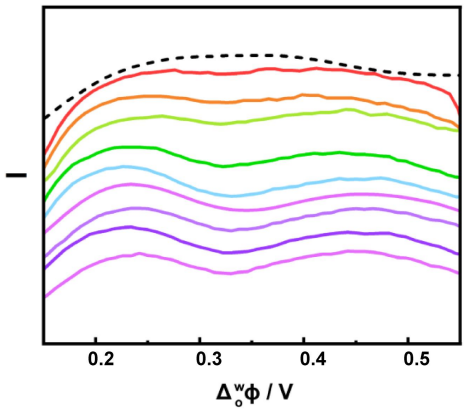


Figure S8. DPV curves obtained at micropipette filled with PVC-TAC gel under different concentrations of K^+.

10. Selectivity tests.

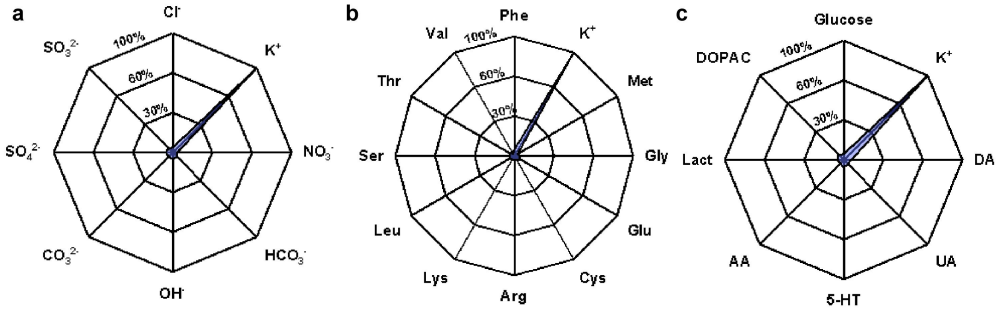


Figure S9. Selectivity tests obtained at ILG-TAC electrode for K^+ in the presence of (a) anions (NO_3^-, HCO^{3-}, OH^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-} and Cl^-. Concentrations: 10 μM), (b) amino acids (Phe, Met, Gly, Glu, Cys, Arg, Lys, Leu, Ser, Thr and Val. Concentrations: 10 μM) and (c) biomolecules and neurotransmitters (AA, DA, UA, 5-HT, DOPAC, glucose and lactate. Concentrations: 10 mM for glucose, 10 μM for the others).

11. Competition tests.

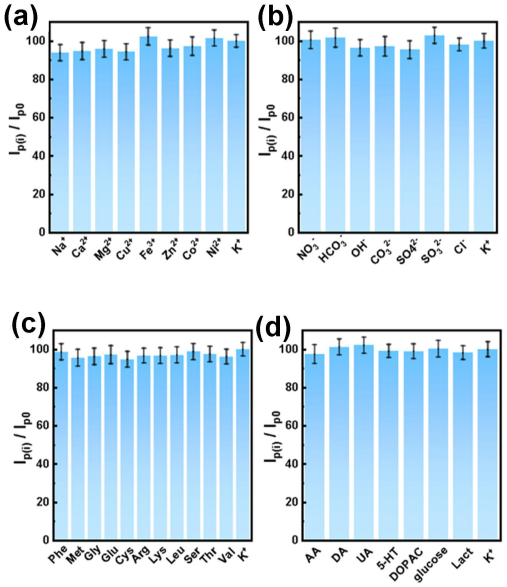


Figure S10. Competition tests for K^+ in the presence of (a) metal cations (Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+} and Ni^{2+}. Concentration: 10 mM for Na^+, 1 mM for Ca^{2+} and Mg^{2+}, and 10 μM for other ions), (b) anions (NO_3^-, HCO^{3-}, OH^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-} and Cl^-. Concentrations: 10 μM), (c) amino acids (Phe, Met, Gly, Glu, Cys, Arg, Lys, Leu, Ser, Thr and Val. Concentrations: 10 μM) and (d) biomolecules and neurotransmitters (AA, DA, UA, 5-HT, DOPAC, glucose and lactate. Concentrations: 10 mM for glucose, 10 μM for the others). $I_{p(i)}$ value represents the peak current generated by 5 mM K^+ and the corresponding interferences. I_{p0} is the peak current generated by 5 mM K^+. S.D=3

12. GFAP staining of coronal brain sections.

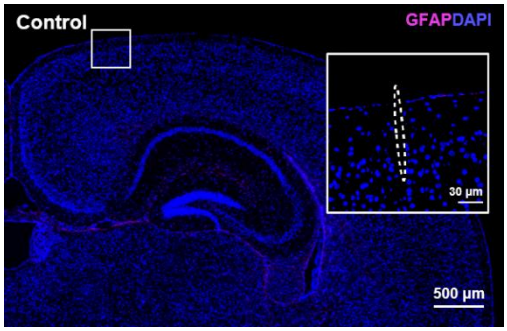


Figure S11. GFAP staining images of coronal brain sections from the rat in the sham-operated group.

13. TTC staining of brain tissue slices.

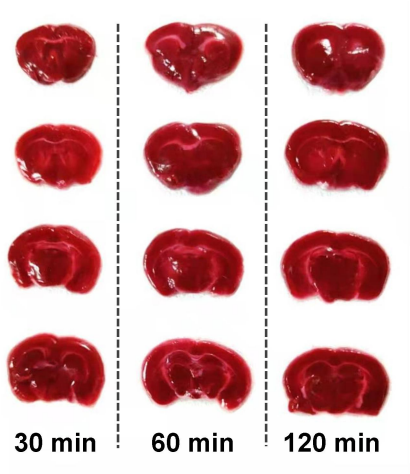


Figure S12. 2,3,5-Triphenyltetrazolium chloride (TTC) staining of brain tissue slices after immersion of the ILG-TAC electrode into the live brain.

14. Electrocardiograms of normal rat and acute hyperkalemia model rat.

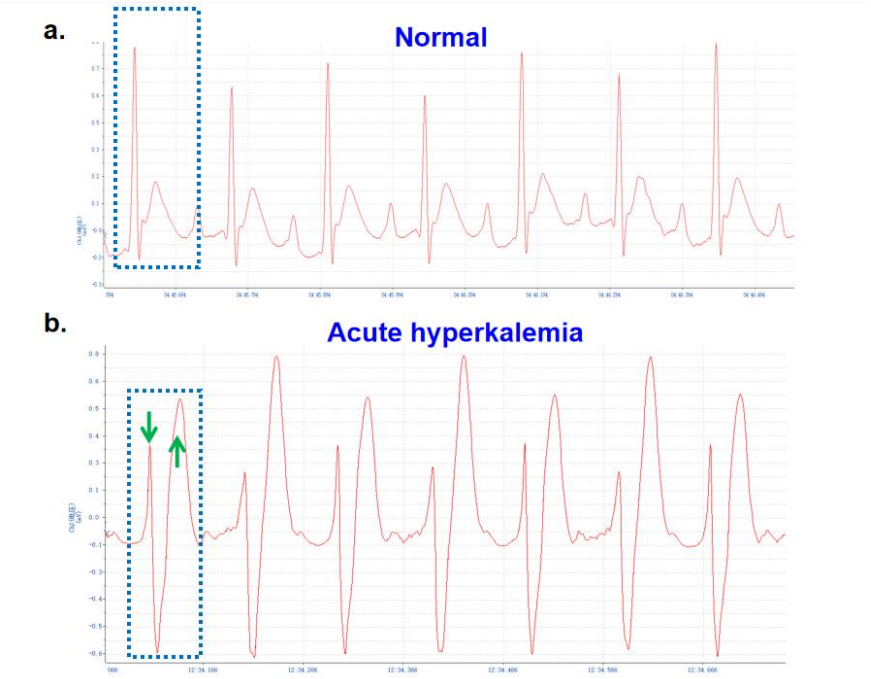


Figure S13. Electrocardiograms of (a) normal and (b) acute hyperkalemia model rats.

16. Electrocardiograms of normal rat and chronic hyperkalemia model rat.

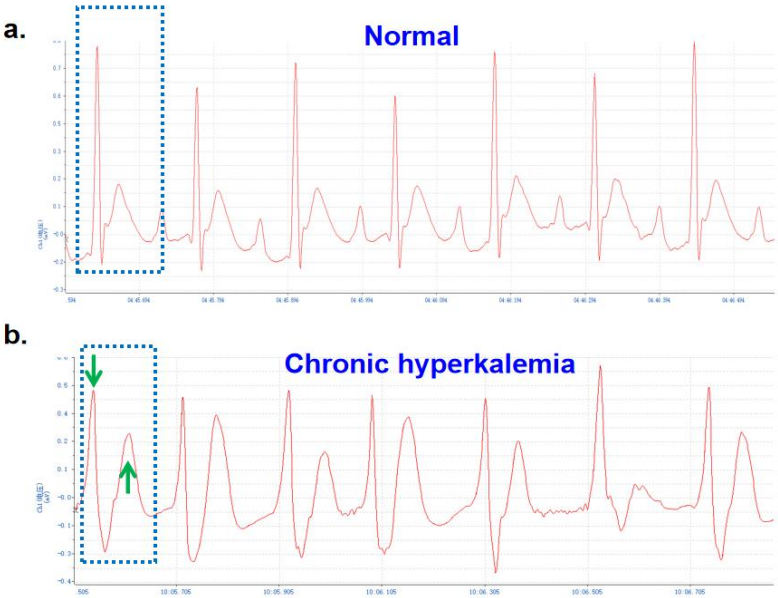


Figure S14. Electrocardiograms of (a) normal and (b) chronic hyperkalemia model rats.