Supplementary Information

Facile Surface Chemical Modification of Single Glass Nanopore and Its Use for Nonenzymatic Detection of Uric Acid

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EXPERIMENTAL SECTION

Chemicals and Materials. Poly(L-histidine) (PLH) hydrochloride (Mw ≥ 5000), hydroxylamine hydrochloride ($NH_2OH·HCl$, 99.999%), 2-thiouracil (2-TU, 99%), uric acid (UA, 99%), L-ascorbic acid (AA, 99%) and D-(+)-glucose (Glu, 99.5%) were purchased from Sigma-Aldrich. Gold(III) chloride tetrahydrate ($HAuCl_4·4H_2O$, 99.0%), potassium chloride (KCl, 99.5%), L-cysteine (LC, 99%) L-lysine (LL, 99%), urea (U, 99%) were obtained from Beijing Chemical Works. All chemicals were used as received and without any further purification. All aqueous solutions were prepared using deionized (DI) water with a resistivity of 18 MΩ·cm.

Glass Capillary Nanopore Fabrication. Quartz glass capillaries (QF100-70-10, Sutter Instrument Co.), with an outer diameter of 1.0 mm and an inner diameter of 0.70 mm, were used for all experiments. Prior to fabrication, the quartz glass capillaries were thoroughly cleaned in a bath of freshly prepared piranha solution (3:1 98% H_2SO_4 : 30% H_2O_2) for ~ 2 h to remove contamination resulting from the production process or transport, storage, and handling process. (Caution: piranha solution is a powerful oxidizing agent and reacts violently with organic compounds. It should be handled with extreme care.) Then these capillaries were rinsed thoroughly with DI water and vacuum drying at 80 °C before use. Glass capillary nanopores were then prepared by pulling the cleaned quartz glass capillaries with a CO_2 laser-based pipet puller (model P-2000, Sutter Instrument Co.) The parameters of the one-line program used to pull quartz glass capillaries were as follows: Heat = 700, Fil = 4, Vel = 40, Del = 175, Pull = 190. The as-prepared glass capillary nanopore tips had inner diameters ranging from 17 to 44 nm, with the mean diameter of 29 nm.

Experimental Setup and Data Acquisition. All ionic current measurements were performed using an Axopatch 200B amplifier (Axon Instruments, USA) in voltage-clamp mode with the Digidata 1440A digitizer (Molecular Devices) and a PC equipped with pCLAMP10.2 software (Molecular Devices). The current−voltage ($I-V$) curves were recorded by sweeping the voltage from −500 to +500 mV. The applied voltage corresponds to the potential of the internal Ag/AgCl electrode versus the external Ag/AgCl electrode (as shown in our previous work).[1] Current−time ($I-t$) curves were recorded upon applying bias

voltage of −200 mV, using a sampling frequency of 200 kHz. The *I*−*V* and *I*−*t* recordings were plotted with pCLAMP10.2 software and Origin8.0.

Functionalization of Single Glass Nanopores. The functionalization process of glass nanopore was illustrated schematically in Fig. 1. The freshly prepared quartz glass nanopores were first modified by physisorption through electrostatic interaction with a layer of positively charged PLH. The glass nanopore was backfilled with 0.01 M KCl solution (buffered with 10 mM HEPES at pH 5.2) unless otherwise indicated, and then immersed into a PLH solution (0.2 mg/mL). A negative cosine voltage (−500 mV, 5 Hz, the stimulus waveform signal was shown in Fig. S4 a) was applied to the nanopore to help the outside PLH infiltrate into the very tip of the glass nanopore and allow the positively charged PLH to physisorb on the negatively charged inner wall of quartz nanopore for 3 min.[2] The quartz nanopore was then immersed into a $HAuCl_4$ (0.1%) bath with a positive cosine voltage applied (+500 mV, 5 Hz, the stimulus waveform signal was shown in Fig. S4 b) for another 3 min. During this process, the negatively charged $AuCl_4^-$ ions would be driven into the nanopore under the oscillating electric field, and attached on the inner wall of nanopore through the "linker" PLH due to its high affinity with Au^{3+}.[3] Subsequently, the quartz nanopore was immersed into $NH_2OH{\cdot}HCl$ solution (20 mM), and applied a cosine voltage (500 mV, 5 Hz, the stimulus waveform signal was shown in Fig. S4 c) to it for 5 min.[3] Finally, the nanopore was immersed into a 2-TU (2 mM) solution with the same cosine voltage applied. After 5 min, the nanopore was moved out from the 2-TU solution and for the follow-up experiments. In addition, the *I*−*V* curves of the functionalization process were recorded in 0.01 M KCl solution (buffered with 10 mM HEPES at pH 5.2). The corresponding current-time traces for every step of the glass nanopore surface modifications were shown as Fig. S5.

Uric Acid Detection by Using 2-TU−Functionalized Glass Nanopore. The functionalized single glass nanopore was dipped into 2 mL of KCl solution (0.01 M, buffered with 10 mM HEPES at pH 5.2) unless otherwise indicated. Then a bias voltage of −200 mV was applied to the nanopore, and 0.2 mL of UA was added each time for the *I*−*t* recording. The concentration of UA was 0.2 mM, which was prepared using 0.01 M KCl with 10 mM HEPES buffer. For the detection of UA in real sample, the serum, which was

supplied by The Second Hospital of Jilin University and collected from a health man, was first treated by centrifugation (10 000 rpm, 10 min); and then 0.2 mL of serum was added into 2 mL of KCl solution (buffered with 10 mM HEPES at pH 5.2) for the *I*−*t* recording upon applying a bias voltage of − 200 mV.

Characterizations: Transmission electron microscopy (TEM) images and energy-dispersive X-ray spectroscopy (EDX) analysis were taken by using an FEI TECNAI F20 EM with an accelerating voltage of 200 kV equipped with an energy dispersive spectrometer. TEM samples were prepared by placing a tip of the glass capillary on a folding grid carefully.

1 H. L. He, X. L. Xu and Y. D. Jin, *Anal. Chem.*, 2014, **86**, 4815.

2 S. Umehara, N. Pourmand, C. D. Webb, R. W. Davis, K. Yasuda and M. Karhanek, *Nano Lett.*, 2006, **6**, 2486.

3 Y. D. Jin and X. H. Gao, *Nat. Nanotechnol.*, 2009, **4**, 571.

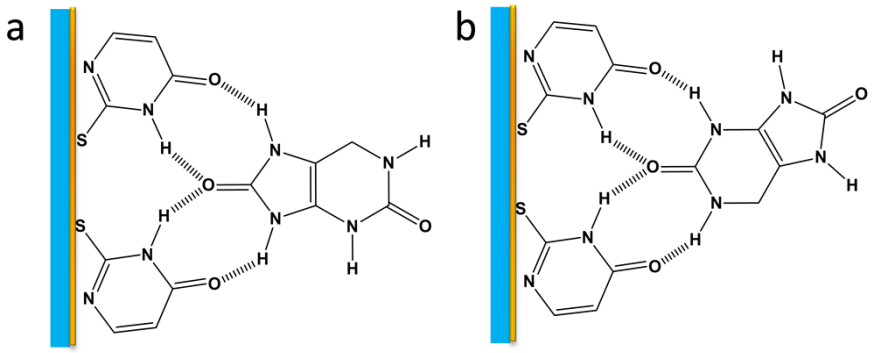

Fig. S1 The possible H-bonding interaction modes of uric acid (UA) with 2-thiouracil (2-TU) that self-assembled on the inner surface of the glass nanopore.

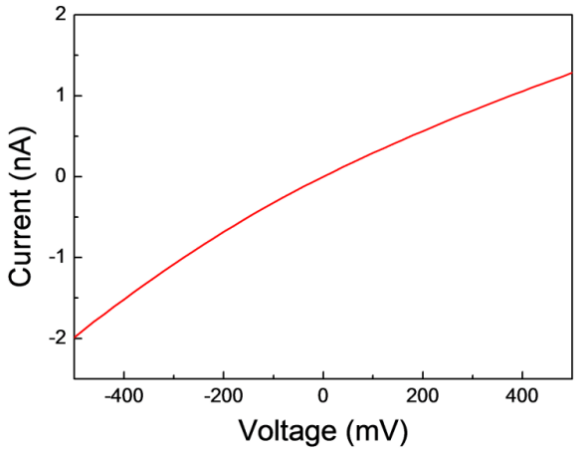

Fig. S2 The typical *I–V* curve of a glass nanopore with pore diameter of ~ 28 nm recorded in 0.01 M KCl (buffered with 10 mM HEPES, at pH 5.2).

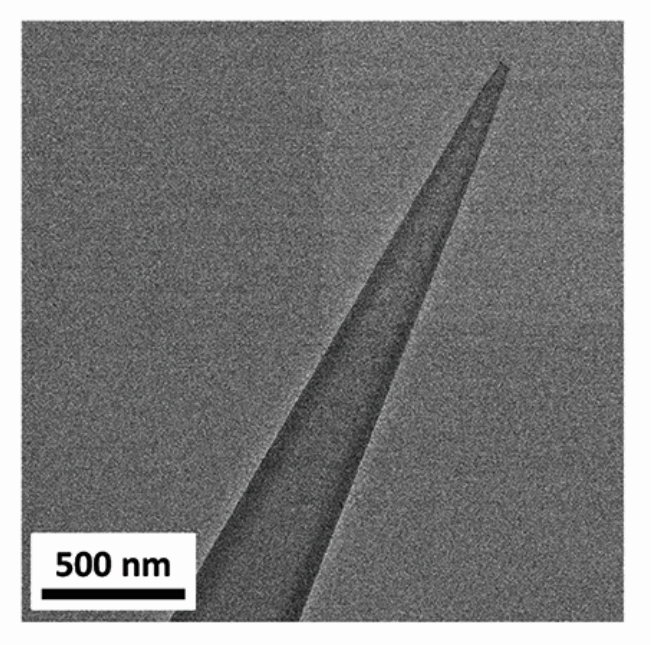

Fig. S3 The TEM image of an unmodified single quartz glass nanopore at low TEM magnification, whose diameter was measured to be ~ 29 nm.

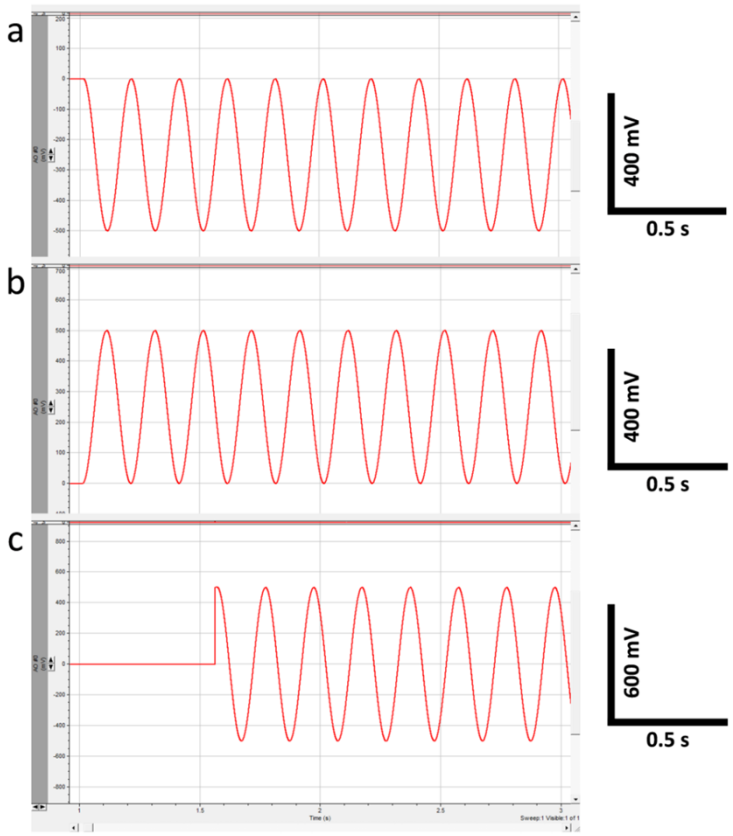

Fig. S4 The applied voltage signals used to modify the glass nanopores. The horizontal ordinate represents time, and the ordinate represents voltage. (a) The stimulus waveform signal of a negative cosine voltage (−500 mV, 5 Hz). (b) The stimulus waveform signal of a positive cosine voltage (+500 mV, 5 Hz). (c) The stimulus waveform signal of a cosine voltage (500 mV, 5 Hz).

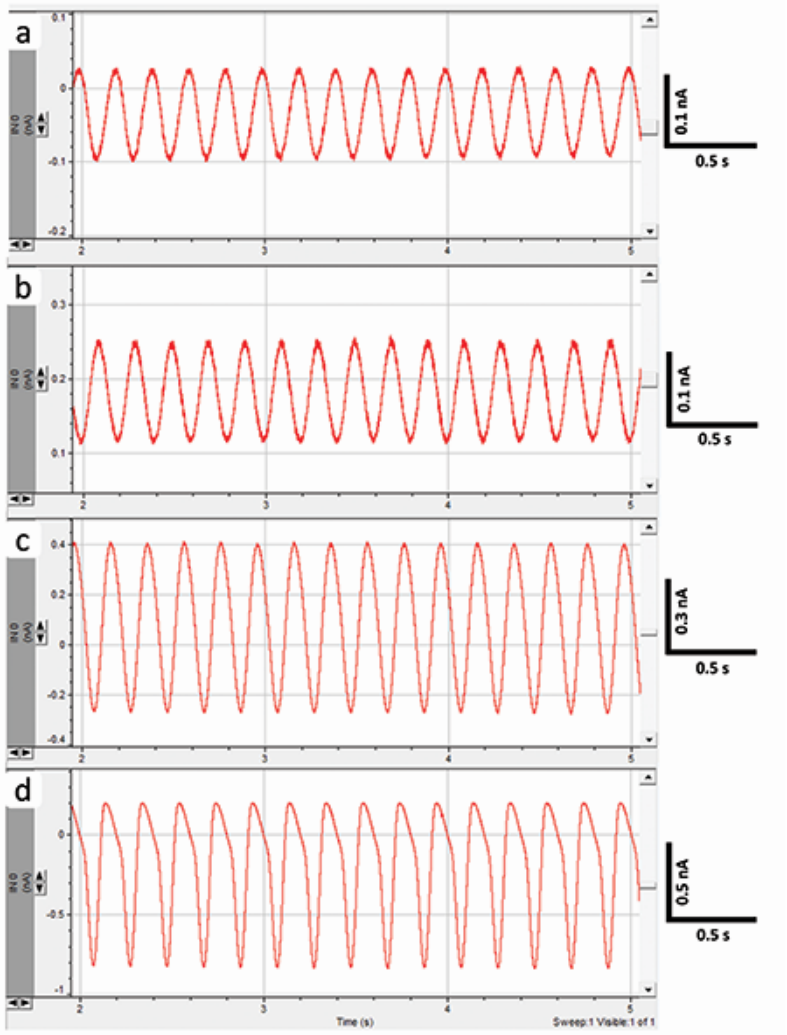

Fig. S5 The current-time (*I*−*t*) traces of the glass nanopore during surface modification processes. The horizontal ordinate represents time, and the ordinate represents current. (a) The corresponding current-time trace of the glass nanopore immersed into a PLH solution with a negative cosine voltage (−500 mV, 5 Hz) applied. (b) The corresponding current-time trace of the PLH absorbed glass nanopore immersed into a $HAuCl_4$ bath with a positive cosine voltage (+500 mV, 5 Hz) applied. (c) The corresponding current-time trace of the glass nanopore immobilized with $AuCl_4^-$ ions immersed into $NH_2OH{\cdot}HCl$ solution with a cosine voltage (500 mV, 5 Hz) applied. (d) The corresponding current-time trace of the gold-decorated glass nanopore immersed into a 2-TU solution with a cosine voltage (500 mV, 5 Hz) applied.

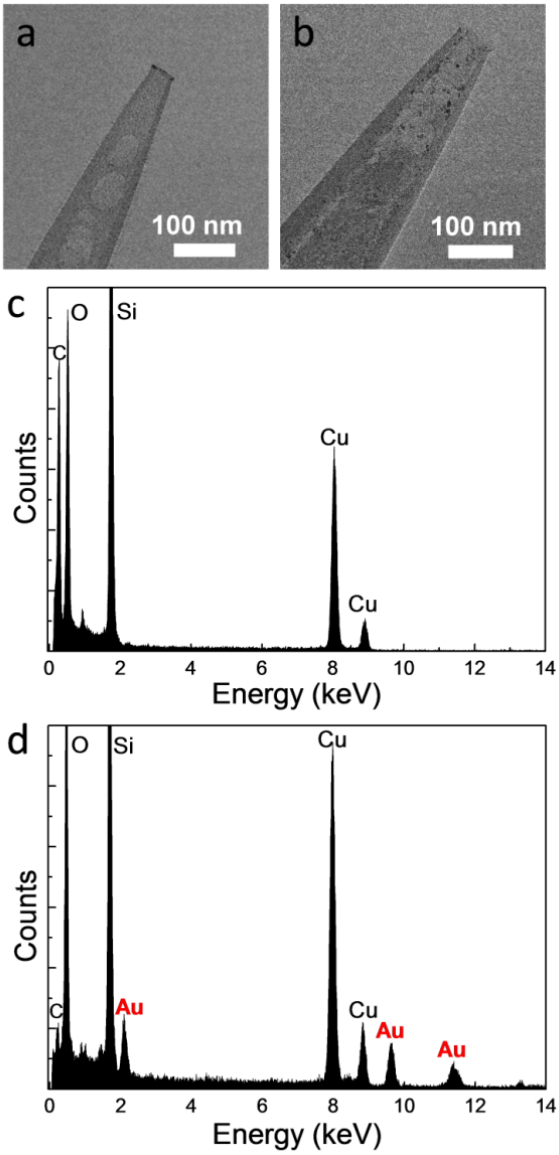

Fig. S6 (a) TEM image of an unmodified single quartz glass nanopore. (b) TEM image of a gold modified glass nanopore. (c) EDX spectrum of the unmodified single glass nanopore. (d) EDX spectrum of the gold modified glass nanopore.

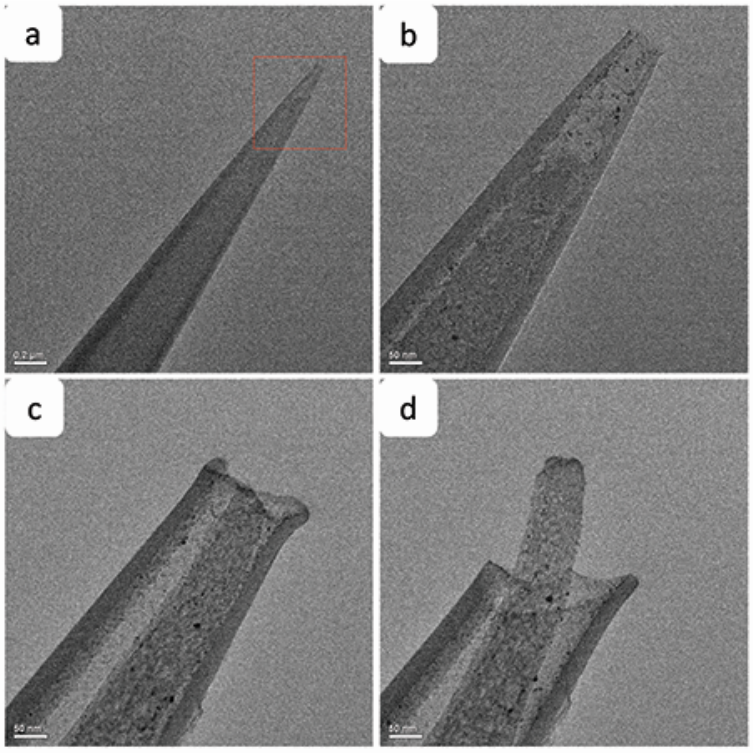

Fig. S7 The TEM images of the gold modified glass nanopore in Fig. S5 b collected under strong electron beam irradiation. Figure (a) was collected under low magnification at the beginning of the TEM measurements; Figure (b) was collected under medium magnification; Figure (c) and (d) were collected under high magnification in one minute.

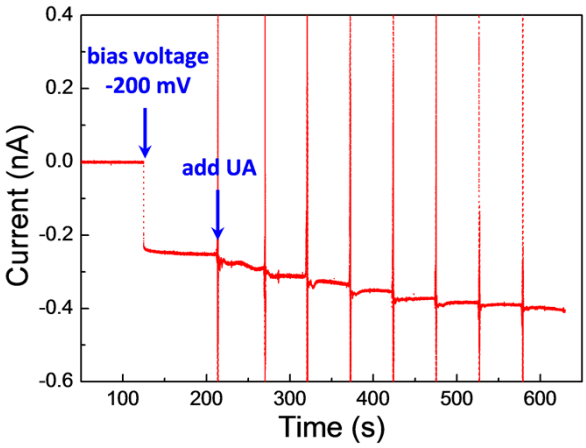

Fig. S8 The typical *I*−*t* plot of the 2-TU functionalized glass nanopore on successive step changes of UA concentration. The functionalized single glass nanopore was dipped into 2 mL of KCl solution (0.01 M, buffered with 10 mM HEPES at pH 5.2). Then a bias voltage of − 200 mV was applied to the nanopore, and 0.2 mL of UA was added each time for the *I*−*t* recording. The concentration of UA added in the bulk solution was 0.2 mM.

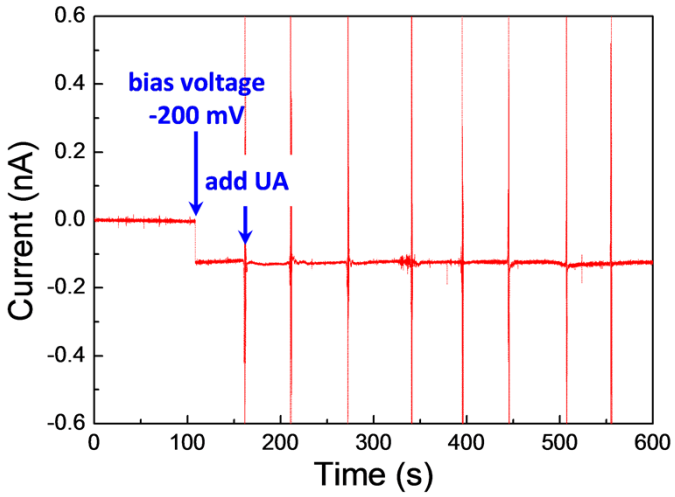

Fig. S9 The typical *I*−*t* plot of the unmodified glass nanopore on successive step changes of UA concentration. The unmodified single glass nanopore was dipped into 2 mL of KCl solution (0.01 M, buffered with 10 mM HEPES at pH 5.2). Then a bias voltage of − 200 mV was applied to the nanopore, and 0.2 mL of UA was added each time for the *I*−*t* recording. The concentration of UA added in the bulk solution was 0.2 mM.

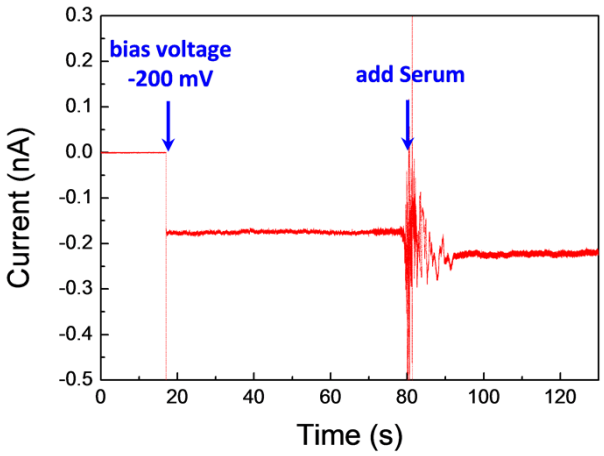

Fig. S10 The *I*–*t* plot of the 2-TU functionalized glass nanopore for the detection of UA in a serum sample.

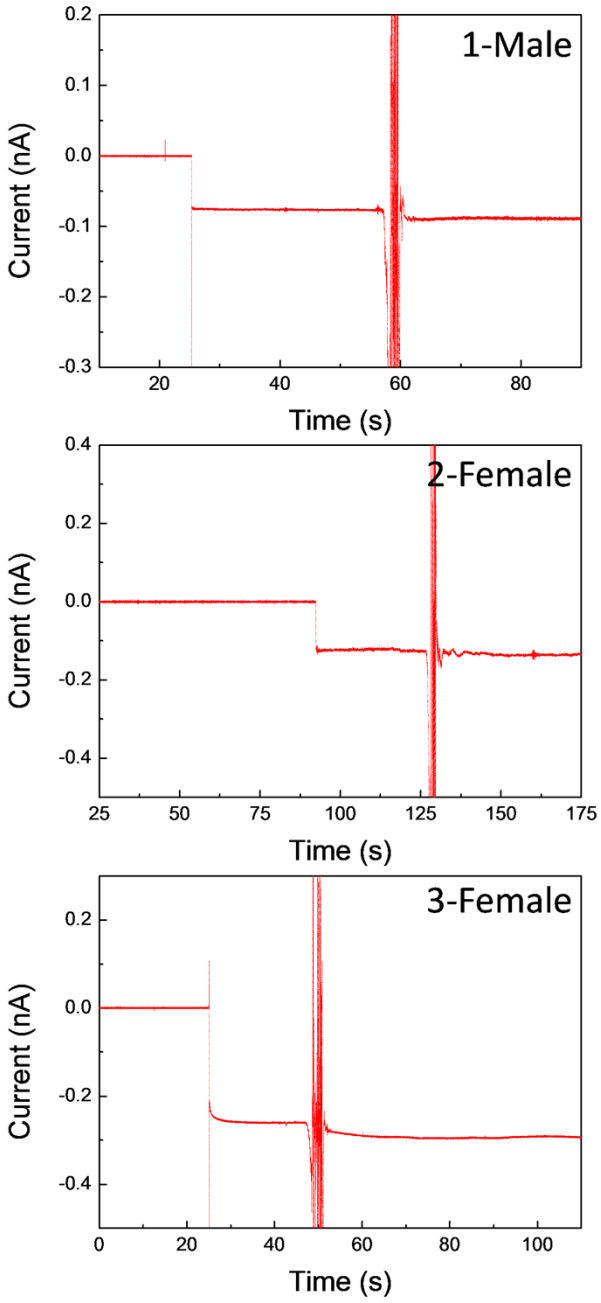

Fig. S11 The *I*−*t* plot of the 2-TU functionalized glass nanopore for the detection of UA in different serum samples. For these three samples detection, 0.1 mL of serum was added into 2 mL of KCl solution (buffered with 10 mM HEPES at pH 5.2) for the *I*−*t* recording upon applying a bias voltage of − 200 mV.

Table 1 The 2-TU−functionalized glass nanopore used for different serum samples detection.

c (UA)	1-Male	2-Female	3-Female
Detected in hospital	424 μM	307 μM	347 μM
Detected by nanopore	443 μM	300 μM	321 μM