Electronic Supplementary Information

Interference-Free and Rapid Electrochemical Lateral-Flow Immunoassay for One-Step Ultrasensitive Detection with Serum

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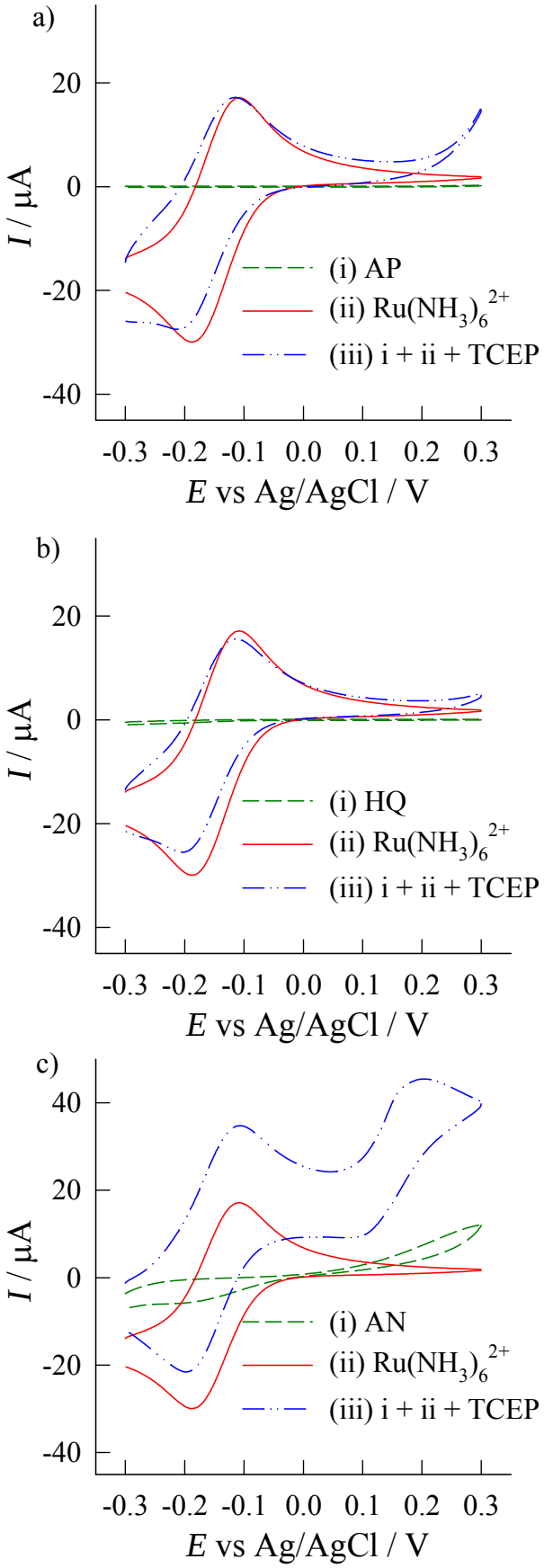

Fig. S1 Cyclic voltammograms that were obtained at bare ITO electrodes (at a scan rate of 20 mV/s) in PBS buffer (pH 6.0) containing either (i) 1.0 mM $Ru(NH_3)_6^{2+}$, (ii) 0.5 mM P, or (iii) 1.0 mM $Ru(NH_3)_6^{2+}$, 0.5 mM P, and 2.0 mM TCEP after argon pursing for 10 min. P was a) AP, b) HQ, and c) AN. Curve i of Figure S1a and S1b shows that redox reactions of AP and HQ are slow at ITO electrodes. The cyclic voltammogram in a solution containing AP (or HQ), $Ru(NH_3)_6^{2+}$, and TCEP [curve iii of Figure S1a (or S1b)] is similar to that in a solution containing $Ru(NH_3)_6^{2+}$ only [curve ii of Figure S1a (or S1b)]. Anodic currents in the cyclic voltammogram in a solution containing AN, $Ru(NH_3)_6^{2+}$, and TCEP [curve iii of Figure S1c)] are much higher than those in a solution containing $Ru(NH_3)_6^{2+}$ only (curve ii of Figure S1c).

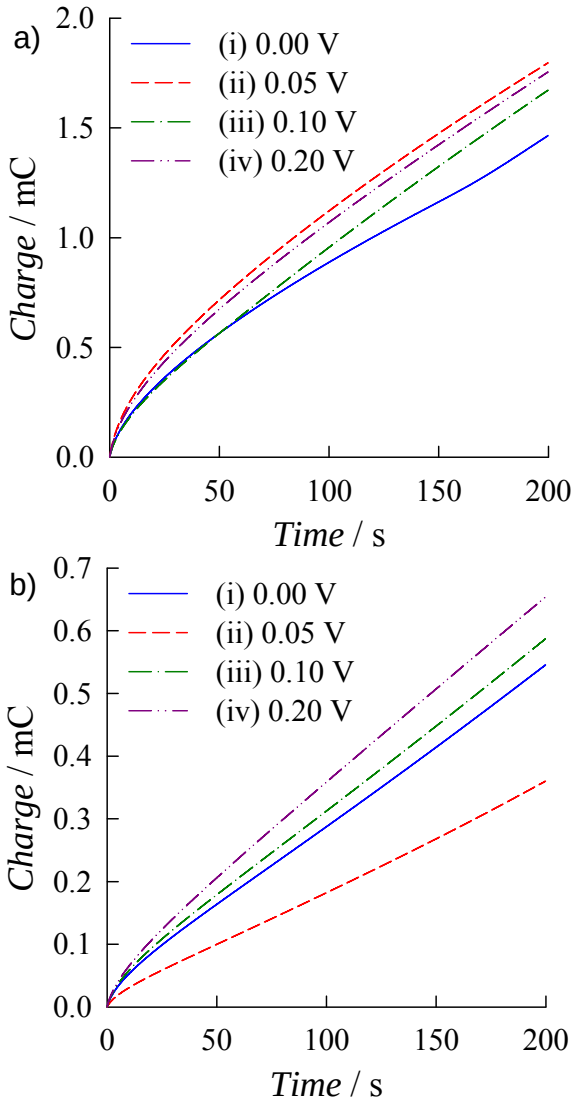

Fig. S2 Chronocoulograms obtained at different potentials at an ITO electrode a) in PBS buffer (pH 6.0) containing 1.0 mM $Ru(NH_3)_6^{3+}$, 0.10 mM AN, and 2.0 mM TCEP or b) in PBS buffer containing 1.0 mM $Ru(NH_3)_6^{3+}$ and 2.0 mM TCEP.

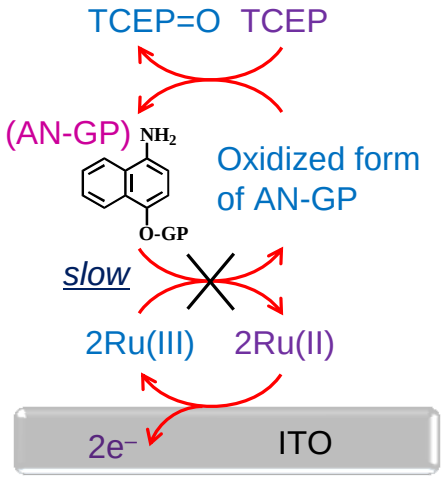

Fig. S3 Schematic representation of ECC redox cycling of AN-GP.

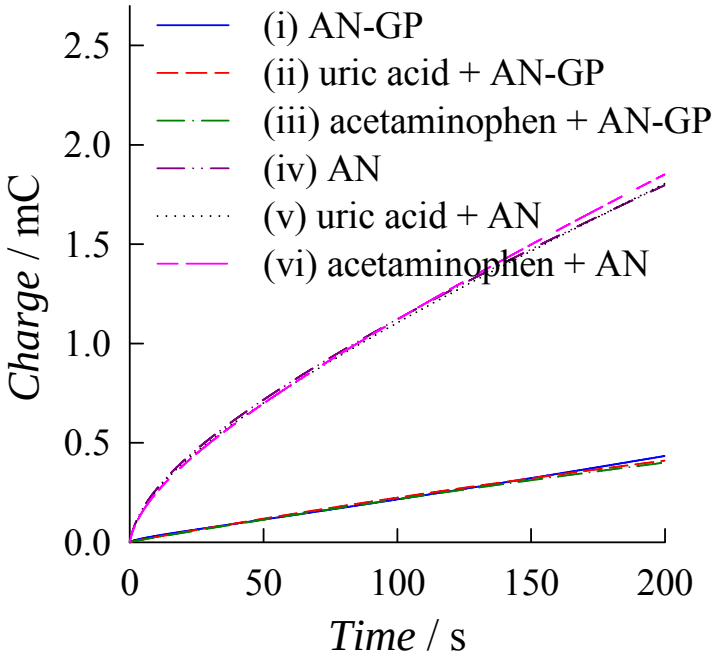

Fig. S4 Chronocoulograms obtained at 0.05 V at ITO electrodes in PBS buffer (pH 6.0) containing 1.0 mM $Ru(NH_3)_6^{3+}$, 2.0 mM TCEP, and either (i) 1.0 mM AN-GP, (ii) 0.1 mM uric acid and 1.0 mM AN-GP, (iii) 0.1 mM acetaminophen and 1.0 mM AN-GP, (iv) 0.1 mM AN, (v) 0.1 mM uric acid and 0.1 mM AN, or (vi) 0.1 mM acetaminophen and 0.1 mM AN.

Fig. S5 Photograph of an electrochemical lateral-flow immunostrip.

Table S1 Comparison between the concentration obtained from the commercial instrument and the concentration obtained from the developed lateral-flow immunoassay.

	Concentration from commercial instrument (ng/mL)	Meaured charge at 60 s (μC) (three measurements)	Calculated approximate concentration (ng/mL)
Sample 1	0.000	4.8 3.4 3.5	0.0004
Sample 2	0.012	7.0 5.9 8.1	0.004
Sample 3	0.050	11 9.1 8.4	0.014
Sample 4	0.644	14 13 13	0.1
Sample 5	8.919	76 57 77	> 100
Sample 6	16.463	100 64 76	> 100

Synthesis of 4-amino-1-napthyl β-D-galactopyranoside

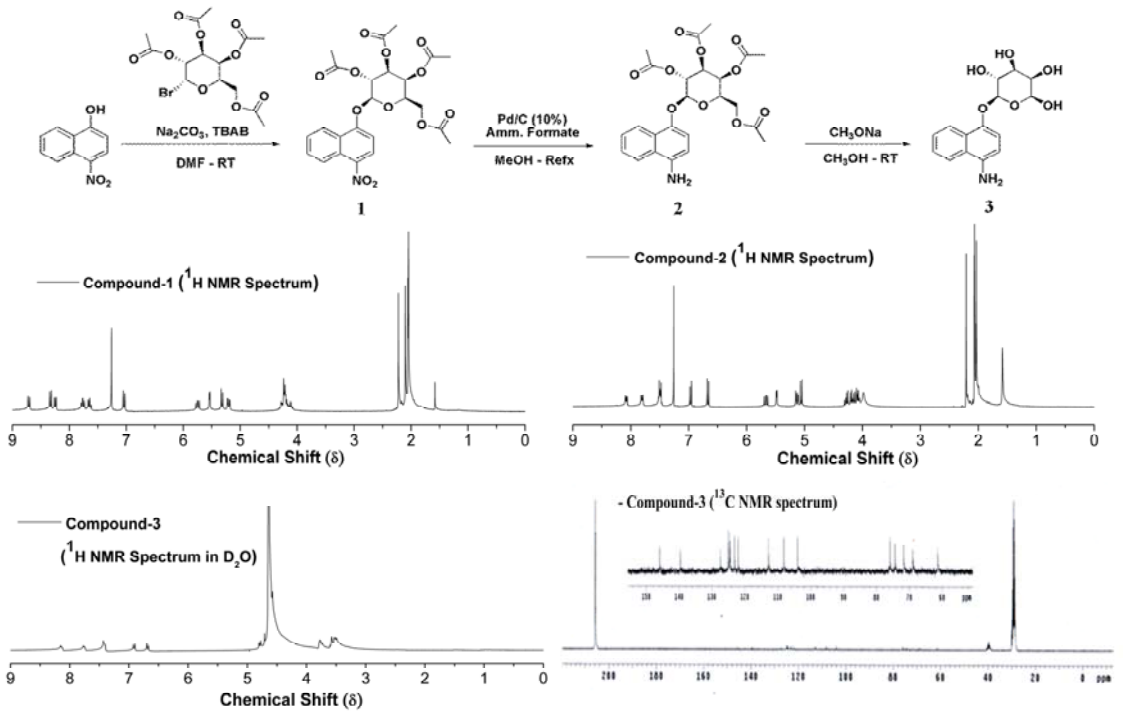

Fig. S6 Synthetic procedure of AN-GP and characterization of two intermediates (the compound 1 and 2) and AN-GP (the compound 3) via ^{1}H NMR and further confirmation of compound 3 via ^{13}C NMR.

The compound 1 was synthesized from the phase transfer-catalyzed reaction between 4-nitro-1-napthol and 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide in the presence of sodium carbonate, tetrabutylammonium bromide and DMF. The presence of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside ring in the compound 1 was confirmed by NMR analysis, and the alkyl and acetyl protons were appeared in the range of 4.10 ~ 5.80 ppm and 2.00 ~ 2.30 ppm, respectively. The nitro group in the compound 1 was reduced to amino group by treating with 10% palladium/carbon and ammonium formate in methanol to yield the compound 2. The NMR analysis of the compound 2 indicates that the new amino protons were appeared at 3.99 ppm. Finally, the deacetylation of the compound 2 using sodium methoxide and methanol offered the compound 3. The NMR analysis of the compound 3 in D_2O or DMSO-d^6 clearly showed the absence of acetyl groups, which suggests that all four acetyl groups were successfully removed. The aryl protons of the compound 3 were clearly found in down filed of the NMR spectrum, but the aliphatic protons were merged with NMR solvent (D_2O or DMSO-d^6) peak. To confirm the aliphatic protons, we took the NMR in mixed solvent (Acetone-d^6 and few drops of DMSO-d^6) system, and the same was nicely found in the new solvent system. The detailed experimental procedure and NMR data of the all compounds were described below.

Synthesis of 4-nitro-1-napthyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (the compound 1). To a stirred solution of 4-nitro-1-napthol (3.5 g, 18.5 mmol) in anhydrous *N*,*N*-dimethylformamide (70 mL) was added sodium carbonate (4.2 g, 40 mmol) and the stirring was continued for 10 min at room temperature. Afterward, 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (8.2 g, 20 mmol) and tetrabutylammonium bromide (50 mg) were added and stirred for overnight. The completion of the reaction was confirmed by thin layer chromatography (TLC), and the mixture was then poured in 100 mL of cold water. The solution was stirred for 30 min and then extracted with ethyl acetate three times (3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and removed by rotary evaporation. The crude product was purified by column chromatography (SiO_2, hexane:ethyl acetate (60:40)) to afford the compound 1 as a yellow solid. The yield was 6.1 g (64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.68 (d, 1 H), 8.31 (d, 1 H), 8.23 (d, 1 H), 7.74 (t, 1 H), 7.62 (t, 1 H), 7.04 (d, 1 H), 5.74 (dd, 1 H), 5.53 (d, 1 H), 5.33 (d, 1 H), 5.21 (dd, 1 H), 4.14-4.30 (m, 3 H), 2.12 (s, 3 H), 2.09 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H).

Synthesis of 4-amino-1-napthyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (the compound 2). The compound 1 (5.0 g, 9.7 mmol) was dissolved in anhydrous methanol (80 mL) and stirred at room temperature. To the stirred solution, 0.5 g of 10% palladium on carbon powder (dry basis) and ammonium formate (3.2 g, 50 mmol) were added, and the mixture was heated to reflux. The reaction progress was monitored by TLC. After 4 h, the completion of the reaction was confirmed, and the mixture was then cooled to room temperature. The black precipitate (10% Pd/C) was filtered off, and the filtrate was concentrated using rotary evaporator. The residue was dissolved into 100 mL of ethyl acetate and washed with water (100 mL) and then brine solution (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated on a rotary evaporator. The crude product was purified by column chromatography (SiO_2, hexane:ethyl acetate (30:70)) to afford compound 2 as a light brown color solid. The yield was 2.8 g (59%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.02-8.12 (m, 1 H), 7.78-7.84 (m, 1 H), 7.46-7.54 (m, 2 H), 6.97 (d, 1 H), 6.67 (d, 1 H), 5.67 (dd, 1 H), 5.48 (d, 1 H), 5.13 (dd, 1 H), 5.06 (d, 1 H), 4.04-4.32 (m, 3 H), 3.99 (brd s, 2 H), 2.21 (s, 3 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H).

Synthesis of 4-amino-1-napthyl β-D-galactopyranoside (the compound 3). The compound 2 (2.0 g, 4 mmol) was dissolved in anhydrous methanol (30 mL) and stirred at room temperature under argon atmosphere. To the stirred solution, 50 mg of sodium methoxide was added. After 10 min, the formation of gray color precipitate was noted and the solution was continuously stirred for additional 10 min. The completion of the reaction was confirmed by TLC, and the mixture was then concentrated by rotary evaporation to dryness. The solid material was successively washed with methanol (25

mL), acetone (50 mL), ethyl acetate (50 mL) and diethyl ether (50 mL). The solid material was dried under vacuum to afford pure product 3 as a light gray color solid. The yield was 1.1 g (86%). ^{1}H NMR (300 MHz, Acetone-d^{6}:few drops of DMSO-d^{6}): δ (ppm) 8.36-8.46 (m, 1 H), 7.98-8.08 (m, 1 H), 7.34-7.48 (m, 2 H), 7.11 (d, 1 H), 6.69 (d, 1 H), 4.88-5.04 (brd s, 2 H), 4.81 (d, 1 H), 4.14-4.24 (m, 1 H), 3.84-98 (m, 2 H), 3.54-3.82 (m, 3 H); ^{13}C NMR (75 MHz, Acetone-d^{6}: few drops DMSO-d^{6}): δ (ppm) 145.9, 139.6, 127.4, 125.1, 124.6, 124.5, 123.1, 122.1, 112.8, 108.2, 104.0, 75.8, 74.3, 71.8, 69.0, 61.4.