# Uric Acid Electrochemical Biosensor Based on Laser-Induced Graphene Electrode Modified with Honey-mediated Nanocomposite of Reduced Graphene Oxide and Bimetallic Silver-Cobalt

## *Supplementary Information*

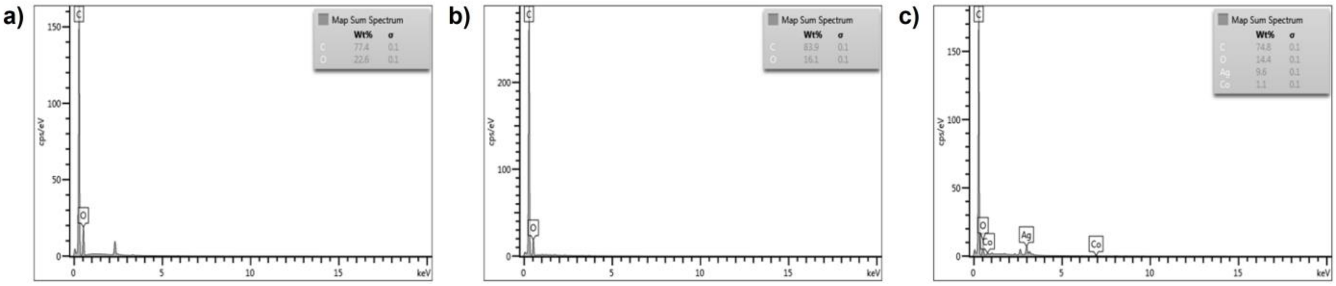

**Figure S1.** EDX analysis of a) GO, b) rGO, and c) rGO/AgCo nanocomposite

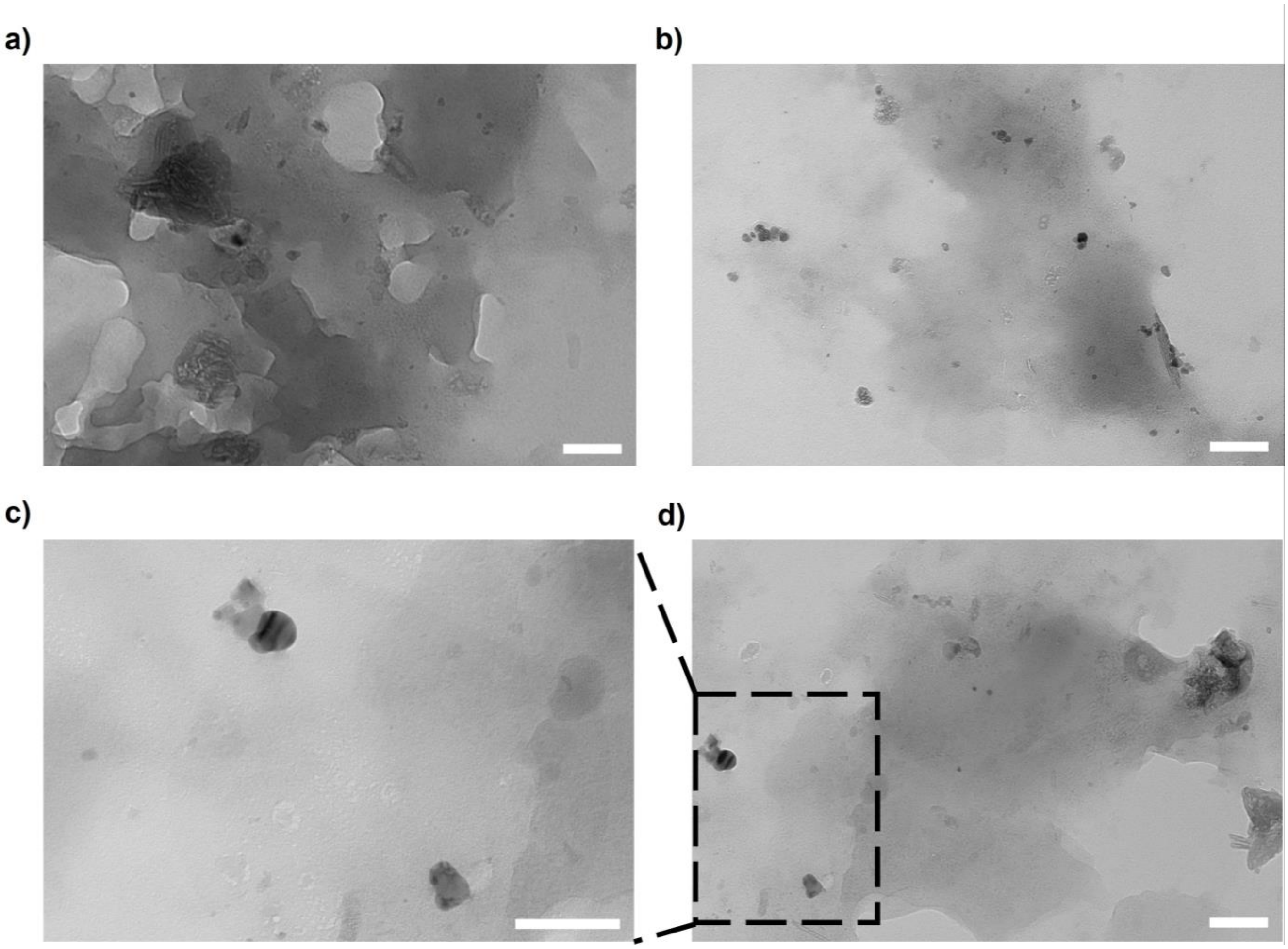

**Figure S2.** TEM analysis of a) GO, b) rGO, c and d) rGO/AgCo nanocomposite (scale bar: 100 nm)

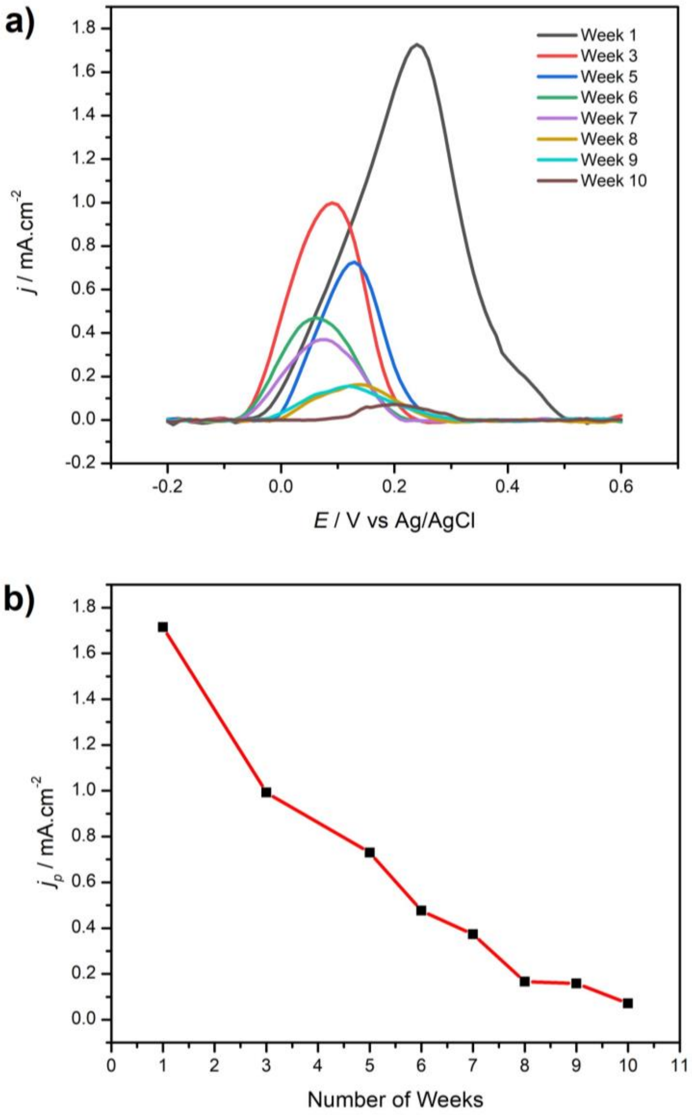

**Figure S3.** Long-term measurement of LIG/rGO/AgCo electrode for UA detection for 2 months, showing the a) DPV test result and the corresponding b) calibration curve.

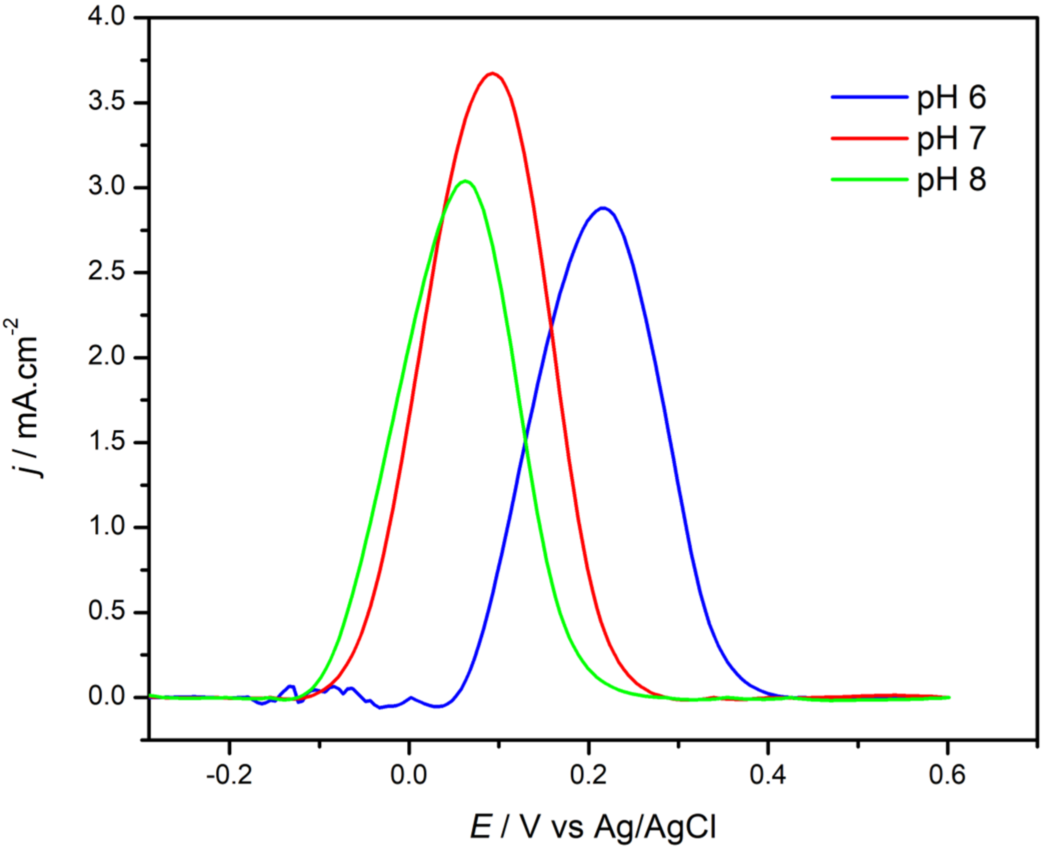

**Figure S4.** DPV measurements of LIG/rGO/AgCo electrode for the detection of 1 mM UA under different electrolyte pH values (6.0, 7.0, and 8.0)

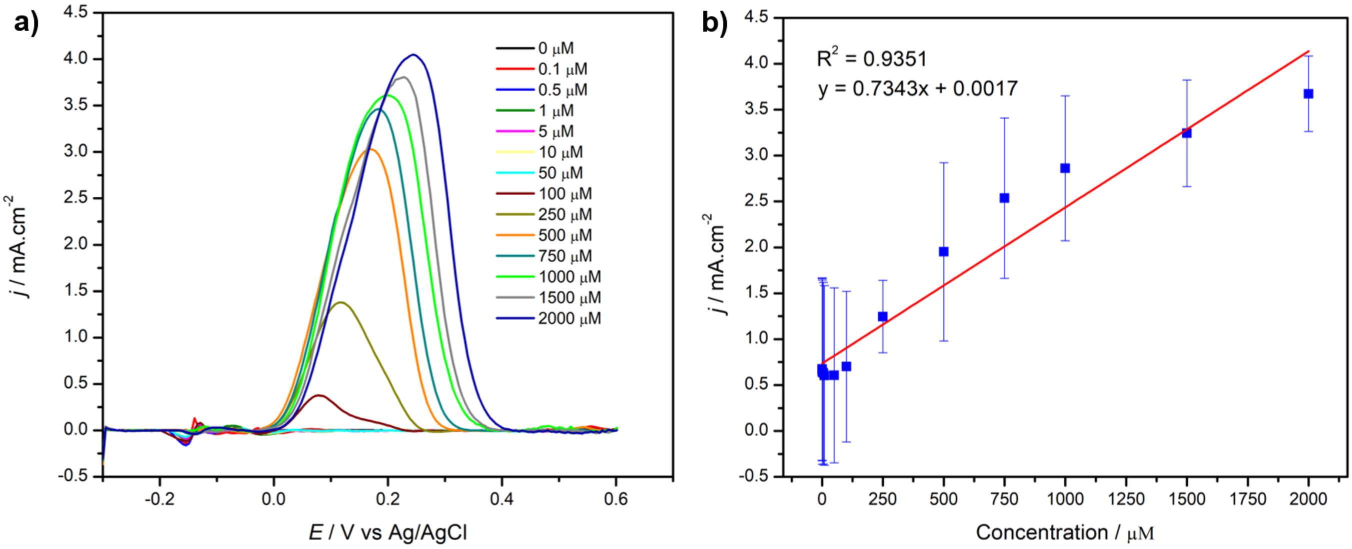

**Figure S5.** DPV measurements of LIG/rGO/AgCo electrode for synthetic urine spiked with UA along with the corresponding d) calibration curve

## Supplementary Note 1: Characterization of Honey as Reducing Agent

Characterization of honey was first conducted to confirm its chemical composition and reducing potential, as evidenced by the FTIR spectrum shown in **Figure S6**. The spectrum reveals several major bands indicative of honey's role as a reducing agent in nanocomposite synthesis. The peaks at 3419 cm$^{-1}$ and 2958 cm$^{-1}$ correspond to O–H and C–H stretching vibration, respectively. These O–H stretching vibration is the typical characteristics originated from the –OH (hydroxyl) groups contained in the water compound of honey. The hydroxyl groups are highly polar and easily attract metals, resulting in the reduction of metal[1].

Furthermore, the C–H stretching vibration originated from the carbohydrates compound of honey. Additionally, the peak at 1639 cm$^{-1}$ is attributed to carbonyl (C=O), which probably correspond to water and some other protein molecules[2]. The small fingerprint region between 1381–1192 cm$^{-1}$ reflects transmission from monosaccharides (fructose, glucose) and disaccharides (sucrose), which are capable to serve as electron donors for metal ion reduction during nanocomposite formation[3]. Additionally, the band at 1058 cm$^{-1}$ specifically indicates fructose compound[3]. In overall, the FTIR spectrum confirmed that the honey contains sufficient hydroxyl, carbonyl, as well as carbohydrates compound, which all acted as a reducing agent, highlighting organic components that are capable of facilitating electron transfer. Thereby, supporting the reduction of metal ions into nanoparticles[4–7]. Besides, from different three samples, all the peaks exactly overlap each others, showing the consistent characteristics of the samples.

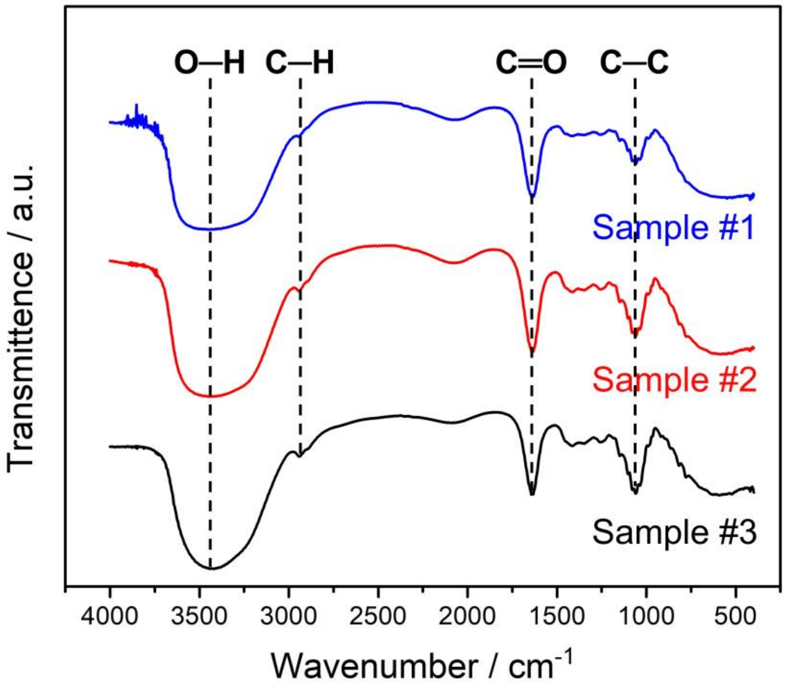

**Figure S6.** FT-IR spectra of natural honey

## Supplementary Note 2: Electrochemical Impedance Analysis of the Bare and the Modified LIG Electrodes

The electrochemical impedance behaviour of the bare and modified electrodes can be explained on a theoretical basis supported by the previous related works. The bare LIG electrodes typically show a larger semicircle in the high-frequency region, reflecting a relatively high charge-transfer resistance (Rct) due to limited electroactive surface area and fewer catalytic sites. Upon modification with nanocomposites, rGO/Ag/Co, Rct is generally expected to decrease, as demonstrated in previous studies [8,9]. This improvement arises from (i) the high conductivity and surface area of rGO, which facilitate electron transport; (ii) the catalytic activity of Ag nanoparticles that accelerate the oxidation of small molecules such as uric acid; and (iii) the redox-active Co/$Co_3O_4$ sites that further lower interfacial barriers.

Accordingly, the Nyquist plot of the modified LIG would be expected to exhibit a smaller semicircle (lower Rct) compared to bare LIG, while the low-frequency tail remains governed by diffusion processes. Similar behaviour has been reported for Ag–rGO and Co–rGO nanocomposites, where electrode modification enhanced electron-transfer kinetics and reduced interfacial resistance.

This theoretical expectation is consistent with previous reports on LIG-based sensors modified with various nanomaterials. For example:

- Adiraju et al. demonstrated that LIG modified with laser-induced fibers and copper phthalocyanine (CuPc) showed lower Rct and enhanced electron-transfer kinetics for nitrite detection, compared to bare LIG[10].
- Nasraoui et al. reported flexible LIG electrodes functionalized with CNT decorated by AuNPs, where modification led to faster electron transfer and decreased Rct in aqueous nitrite detection[11].
- Nugba et al. demonstrated that LIG modification with conductive nanoparticles (CuNPs) enhanced electron transport, resulting in reduced Rct and improved amperometric response[12].
- Han et al. demonstrated that enzyme-based LIG electrodes modified by electroplating PdCu showed improved electrochemical performance, indicating a decrease in Rct and enhanced electron-transfer kinetics[13].

### References:

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