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Supporting Information

Au@ Bovine Serum Albumin Nanoparticles-based acid-resistant Nanozyme Quartz Crystal Microbalance Sensing of Urine Glucose

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Principle of Quartz Crystal Microbalance

QCM sensor was carried out on a quartz crystal microbalance system which was mainly consisted of a controller, a circuit with an oscillator, and a QCM detection chip. The principle of the sensor was based on the Sauerbrey equation ($\Delta m = - C \cdot \frac{\Delta f}{n}$). where C is the sensitivity constant which is related to the properties of quartz. For a 5 MHz crystal, C is sensitivity which means how many ng of material per square centimeter is required to change the resonant frequency by 1MHz and equals to 17.7 ng $(cm^2 \cdot Hz)^{-1}$, and n (1, 3, 5…) is the overtone taken in the test, and in this work, n equals to 3.

Fabrication of HRP+GOx dual enzyme-coated QCM sensor

The preparation procedure for the dual natural enzyme sensor is as follows. The QCM chips were cleaned in the same way as that for the tandem nanozyme. The dual natural enzymes are synthesized by the modification of the active carboxyl group and HRP respectively, on the QCM electrode. Then a given volume of 10 mM 11-Mercaptoundecanoic acid was dissolved in DMSO to form solution, where the QCM electrode is soaked for 30 min and washed with DMSO and distilled water subsequently. The electrode is immersed into a solution composing of 6 mg/mL HRP in a given volume of 500 mM PBS at pH 7 for 1.5 h and then rinsed several times with PBS. Thirdly, the HRP-grafted sensor was modified with 15% (v/v) glutaraldehyde solution for 20 min and rinsed with PBS. Finally, the as-fabricated sensor was soaked in a solution with 3 mg/mL GO*x* and 25 mM PBS at pH 7 for 30 min, washed with PBS and dried under N_2 flow to achieve the final duel-natural enzyme-modified QCM sensor.

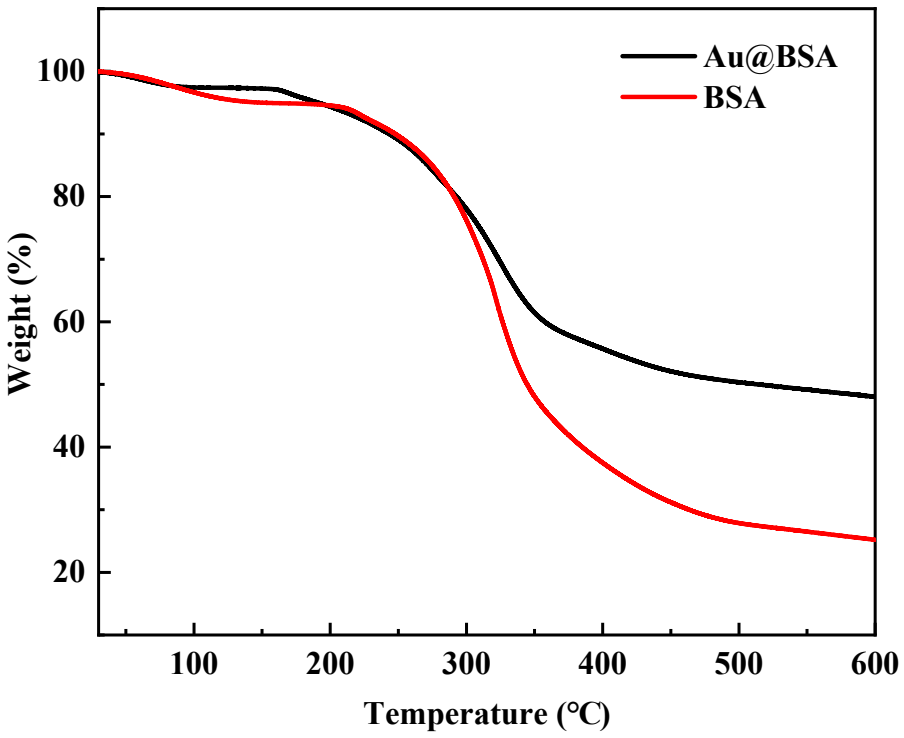

Fig. S1. TGA profile of BSA and Au@BSA NPs.

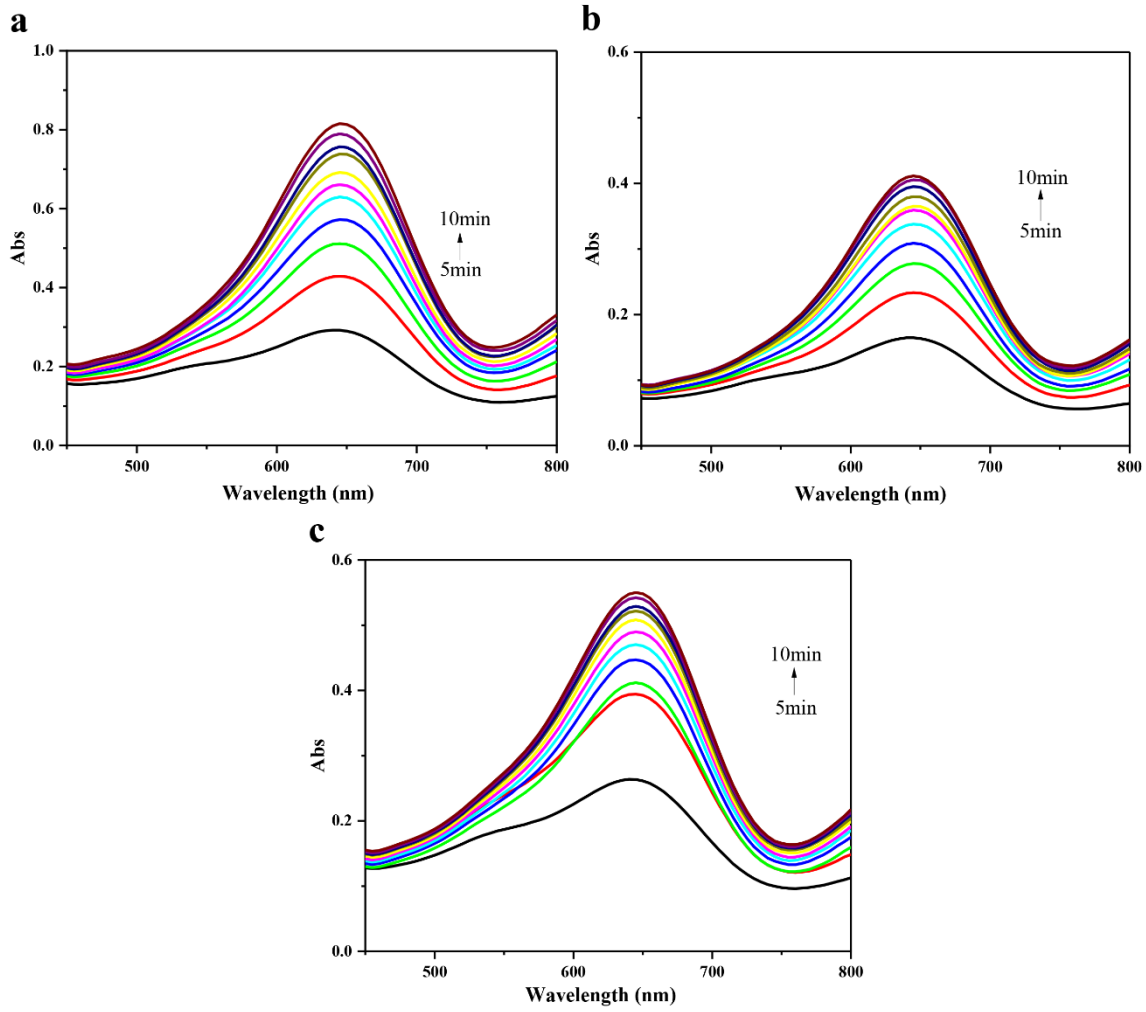

Fig. S2. Absorbance of peroxidase-like (a), GOx-like reaction (b) and the overall reaction catalyzed by tandem nanozyme (c).

The peroxidase-like catalysis activity of the tandem nanozyme was verified using UV-vis absorbance for the reaction between H_2O_2 and TMB, where TMB as the substrate was oxidized by H_2O_2 to cause the change of the characteristic absorbance of $_{OX}$TMB at 650 nm as shown in the Fig. S2(a), which increases with time between 5-10 min, confirming that Au@BSA NPs tandem nanozyme has intrinsic peroxidase-like activity.

Similarly, the GOx-like catalysis activity of the Au@BSA NPs tandem nanozyme was also validated by a two-step reaction, where Au@BSA NPs were incubated firstly with glucose solution for 10 min under ambient conditions and then HRP was added to examine the oxidation extent of TMB as shown in Fig. S2(b). The increasing characteristic absorbance of $_{OX}$TMB at 650 nm proves that the H_2O_2 generated via the oxidization of the glucose in the first step can be consumed by HRP and the Au@BSA NPs have inherent GOx-like activity.

Finally, Au@BSA NPs have also been confirmed as an effective tandem nanozyme as incubated with glucose solution for 10 min under ambient conditions and mixed with TMB as shown in Fig. S2(c). The characteristic absorbance of $_{OX}$TMB at 650 nm between 5-10 min indicates that the nanozyme performs both peroxidase and HRP-like behaviors for the generation of H_2O_2 and the oxidization of TMB, as a tandem nanozyme.

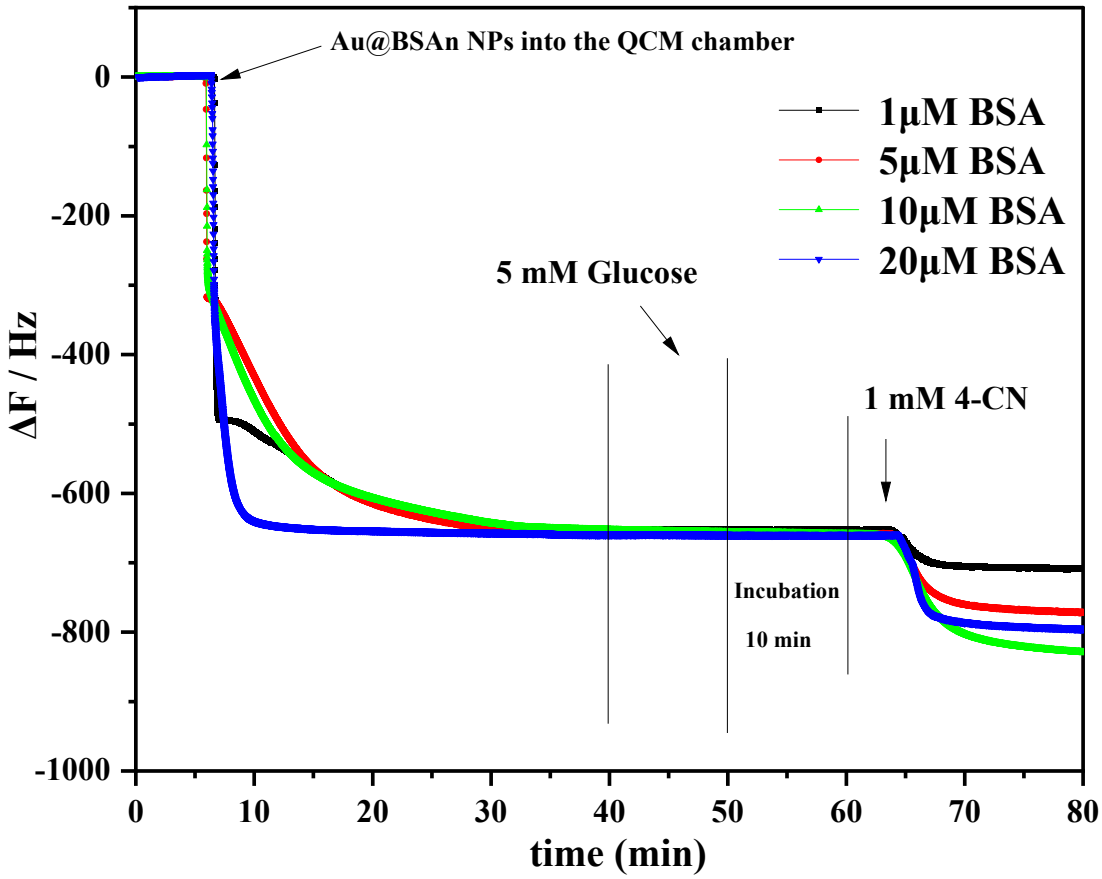

Fig. S3. Dual enzyme-like behavior of Au@BSAn NPs with different Au:BSA ratio.

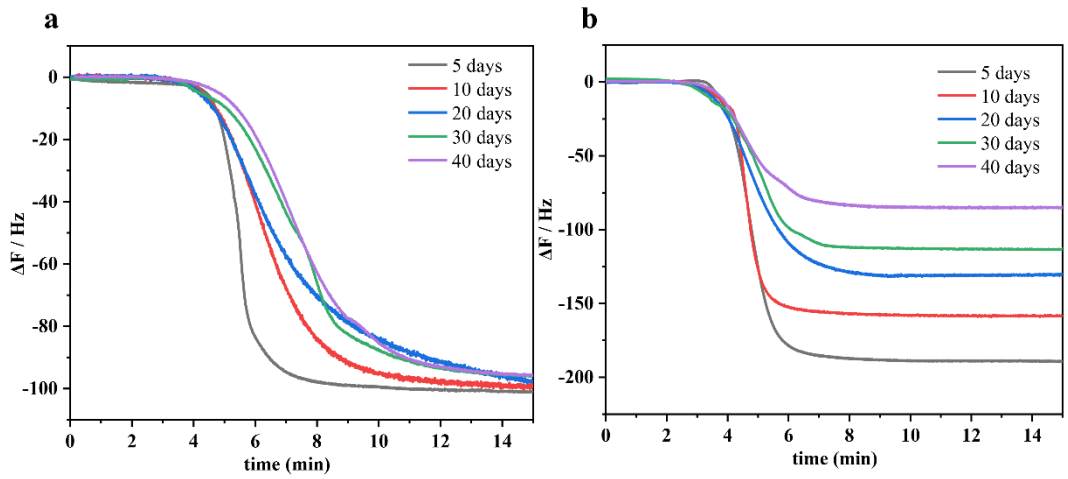

Fig. S4. Storage stability comparison of the Au@BSA NPs tandem nanozyme (a) and the dual HRP+GOx enzyme (b) at 4 °C and pH 7.0 for the detection of 5 mM glucose in solution.